

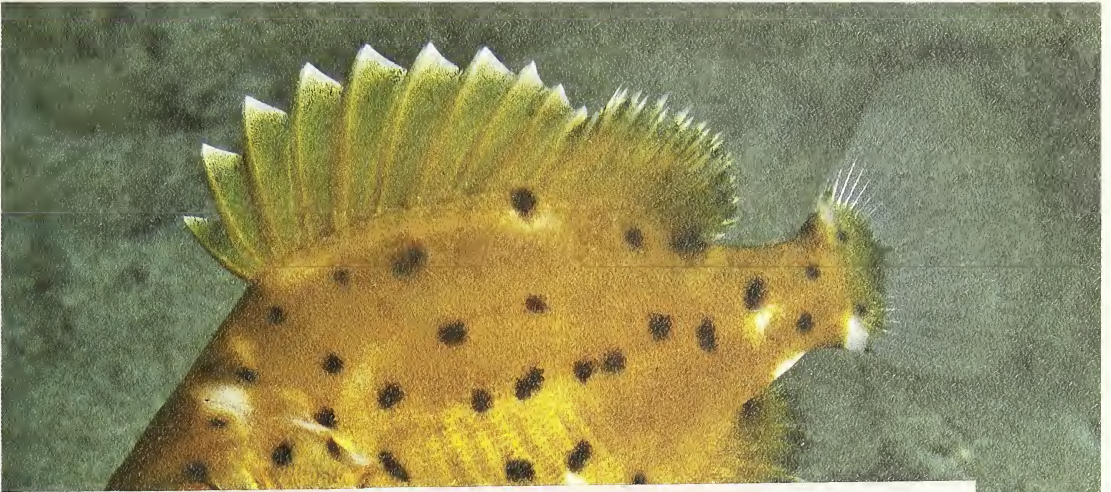
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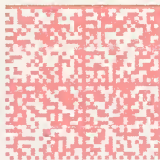
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## Possible Stock Structure of Coastal Bottlenose Dolphins off Baja California and California Revealed by Photo-Identification Research

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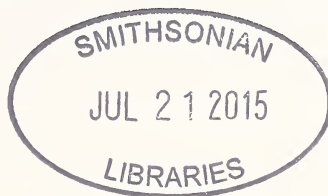
**Abstract.**—Boat-based photo-identification research has been carried out on bottlenose dolphins in eastern North Pacific coastal waters off northern Baja California, Mexico and southern and central California, USA from 1981 to 2001. Within these waters, bottlenose dolphins routinely travel back and forth between coastal locations while generally staying within a narrow corridor extending only 1–2 km from the shore. Inter-area match rates for 616 dolphins photo-identified between 1981–2000 in four California coastal study areas (CCSAs) of Ensenada, San Diego, Orange County and Santa Barbara averaged 76%. To explore possible southern range limits for these dolphins, photo-identification surveys were carried out in the coastal waters off San Quintín, Baja California, Mexico between April–August 1990 ( $n=8$  surveys) and July 1999 to June 2000 ( $n=12$  surveys). The 207 individual dolphins identified off San Quintín were compared to the 616 dolphins identified in the CCSAs. The inter-area match rate between San Quintín and the CCSAs was 3.4% ( $n=7$  dolphins). This low rate contrasts sharply with the much higher average match rate of 76% observed between the CCSAs. These differences in match rates suggest that both a California coastal stock and coastal Northern Baja California stock may exist, with only a limited degree of mixing between them.

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The common bottlenose dolphin (*Tursiops truncatus*) is the most frequently encountered cetacean in the nearshore waters of California and Baja California, Mexico. Two distinct bottlenose dolphin ecotypes occur in these waters: a coastal form that is typically found within 1–2 km of shore (Carretta et al. 1998; Defran and Weller 1999;

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Bearzi 2005) and an offshore form that is distributed in deeper waters, typically greater than a few kilometers from shore (Defran and Weller 1999; Bearzi et al. 2009). Differentiation of these two ecotypes, which are managed as separate stocks by the National Marine Fisheries Service (Carretta et al. 2013), is supported by morphological (Walker 1981; Perrin et al. 2011), photographic (see Shane 1994) and genetic data (Lowther-Thieleking et al. 2014).

The California coastal stock is small, estimated to contain about 450–500 individuals (Dudzik et al. 2006; Carretta et al. 2013) that are distributed between Monterey, California and Ensenada, Baja Mexico (Defran et al. 1999; Hwang et al. 2014), with occasional sightings as far north as San Francisco, California<sup>1</sup>. Photo-identification research has been carried out on the coastal stock off California, and to a lesser extent off Northern Baja California, since the early 1980s. Areas off California and Baja California where photographic data have been collected include: (1) Ensenada, (2) San Diego, (3) Orange County, (4) Santa Monica Bay, (5) Santa Barbara, (6) Monterey Bay and (7) San Francisco Bay (Fig. 1). In general, photo-identification data have shown that California coastal dolphins display little site fidelity to any portion of their distribution (Defran et al. 1999; Hwang et al. 2014). Instead, they routinely travel back-and-forth within their range, on some occasions in excess of 900 km, while at the same time typically staying very near shore (Defran et al. 1999; Hwang et al. 2014).

Records from the nineteenth century suggest that coastal bottlenose dolphins may have once occurred in Monterey Bay and San Francisco Bay (Dall 1873; True 1889; Orr 1963). More recent studies, however, considered the northern range boundary to be located off Los Angeles County up until the early 1980s (Norris and Prescott 1961; Dohl et al. 1981; Leatherwood and Reeves 1982). The 1982–83 El Niño Southern Oscillation (ENSO) dramatically impacted the coastal marine ecosystem off California and Baja. It was during this ENSO event that California coastal stock dolphins extended their northern range back to Monterey Bay (Wells et al. 1990). This northern range extension has persisted to the present day (Riggin and Maldini 2010; Maldini et al. 2010; Cotter et al. 2011) and now extends even further north to San Francisco Bay and most recently, Bodega Bay<sup>1</sup>.

The southern boundary of the California coastal stock is less well known but photo-identification data demonstrate that it extends to at least Ensenada (Defran et al. 1999; Hwang et al. 2014). In this research, boat-based photo-identification surveys of coastal bottlenose dolphins were carried out south of Ensenada off San Quintín Bay, Baja California (Figs. 1 & 2). The goal of this research was twofold: (1) to examine the degree of overlap between coastal dolphins photo-identified off San Quintín and those photo-identified in study areas off Ensenada, San Diego, Orange County, and Santa Barbara, and (2) to use photo-identification data to determine if the southern range of the California coastal stock extended as far south as the San Quintín area.

## Methods

The general design used in this study was the same as our earlier studies that compared independently collected bottlenose dolphin photo-identification catalogs from California and Baja California (Defran et al. 1999; Hwang 2014).

<sup>1</sup> Szczepaniak, I., W. Keener, M. Webber, J. Stern, D. Maldini, M. Cotter, R.H. Defran, M. Rice, G. Campbell, A. Debich, A. Lang, D. Kelly, A. Kesaris, M. Bearzi, K. Causey, and D. Weller. 2013. Bottlenose dolphins return to San Francisco Bay. Poster presented at the 20<sup>th</sup> Biennial Conference on the Biology of Marine Mammals, Dunedin, New Zealand December 9–13.



Fig. 1. Coastal locations where California coastal stock bottlenose dolphins have been photo-identified. Point Conception and Punta Colonet are included to indicate the northern and southern coastal boundaries of the Southern California Bight. Study areas marked with an asterisk indicate those that were compared to San Quintín sightings (Table 1).

### Study Area

This study was conducted in the coastal waters south of San Quintín Bay, Baja California, during two independent study periods: 1) April, June and August 1990,  $n=8$  surveys (Caldwell 1992); and 2) July 1999 to June 2000,  $n=12$  surveys (Morteo et al. 2004). The San Quintín study area was located approximately 376 km south of San Diego and about 200 km south of Ensenada. Within the study area, the survey track extended



Fig. 2. San Quintín study area. Dashed line indicates photo-identification survey route.

32 km southward from a point 8 km east of Azufre Point ( $30^{\circ}23'50''$  N;  $115^{\circ}54'42''$  W) south to Rosario Canon ( $30^{\circ}09'06''$  N;  $115^{\circ}48'27''$  W) (Fig. 2). Most surveys in 1990 began at the Base Camp near El Socorro and extended 18 km south to Rosario Canon. During the 1999–2000 study period, surveys began about 8 km east of Azufre Point and extended 26 km south to Hondo Creek (Fig. 2).

#### *Photo-Identification Surveys and Photographic Data Analysis*

Survey methodology and photo-identification analysis procedures employed during both study periods in San Quintín followed those used previously in the Ensenada, San Diego, Orange County and Santa Barbara study areas, hereafter referred to as California coastal study areas (CCSAs). Detailed descriptions of these procedures are provided

Table 1. Summary information on survey effort, study period, photographic data, and data sources for all study areas<sup>a</sup>.

| Study area    | Number of surveys<br>(complete, partial) | Study period                                          | Number of dolphins<br>identified |
|---------------|------------------------------------------|-------------------------------------------------------|----------------------------------|
| San Quintin   | 20 (20, 0)                               | 1990 <sup>1</sup> , 1999–2000 <sup>2</sup>            | 207                              |
| Ensenada      | 23 (23, 0)                               | 1985–1986 <sup>3</sup> , 1999–2000 <sup>4</sup>       | 129                              |
| San Diego     | 241 (157, 84)                            | 1981–1989 <sup>5</sup> , 1996–1999 <sup>6&amp;7</sup> | 518                              |
| Santa Barbara | 73 (55, 18)                              | 1987 & 1989 <sup>3</sup> , 1998–1999 <sup>7</sup>     | 213                              |

Data sources: <sup>1</sup> Caldwell (1992), <sup>2</sup> Morteo et al. (2004), <sup>3</sup> Defran et al. (1999), <sup>4</sup> Guzón-Zatarain (2002), <sup>5</sup> Defran and Weller (1999), <sup>6</sup> Dudzik (1999), <sup>7</sup> Lang (2002). <sup>a</sup> Some numbers differ from those given in original data sources due to refinement and revision of the dataset over time and the elimination of sightings not meeting the specified photographic quality criteria.

elsewhere (Caldwell 1992; Defran and Weller 1999; Defran et al. 1999; Dudzik 1999; Lang 2002; Morteo et al. 2004) but are briefly described here. Photographic surveys involved slow travel in small boats while moving parallel to the coast and outside the surf line; generally within 500–750 m of shore and corresponding to water depths between 4 m to 10 m. Surveys were conducted in sea state and visibility conditions adequate for finding and photographing dolphins. Although past data demonstrated that most coastal bottlenose dolphins are typically found within 500 m of the shore (Hanson and Defran 1993; Defran and Weller 1999; Bearzi 2005; Carretta et al. 2013), two or more observers, nevertheless, visually searched the area from the shore to ~ 2 km offshore to ensure complete coverage of coastal waters. Once a group of dolphins was sighted, initial estimates of group size, as well as information on time, location, environmental conditions and behavior were recorded.

Following initial estimates of group size, the survey vessel maneuvered to a distance from the dolphins suitable for photo-identification. Thirty-five millimeter SLR film cameras equipped with telephoto lenses were used to photograph all dolphins (marked and unmarked) within a group. Initial estimates of group size were revised as necessary, and contact with the group was maintained until photographic effort was completed, or dolphins began exhibiting avoidance behavior. Identical procedures were repeated as the vessel resumed travel on the predetermined survey route and as additional dolphin groups were encountered.

The best quality photograph of every dolphin was scanned and converted into a high-resolution digital image. Of these, only high quality photographs of dorsal fins with two or more distinctive dorsal fin notches were used for analysis. Distinctive dorsal fins were those that had sufficient notching on the leading or trailing edge such that they could be matched to high quality dorsal fin photographs from other sightings (Urian and Wells 1996; Defran and Weller 1999; Defran et al. 1999; Mazzoil et al. 2004). Only unambiguous matches were accepted as resightings (i.e., a re-identification of a previously identified individual). Dorsal fin images from selected CCSAs (marked with an asterisk in Fig. 1) were analyzed and maintained in the Cetacean Behavior Laboratory at San Diego State University. The combined photo-identification catalog for the CCSAs consisted of 616 individuals identified during two sample periods: (1) 1981 to 1989, and (2) 1996 to 2000. Table 1 provides a summary of survey effort, study period, photographic data and data sources for each of the CCSAs.

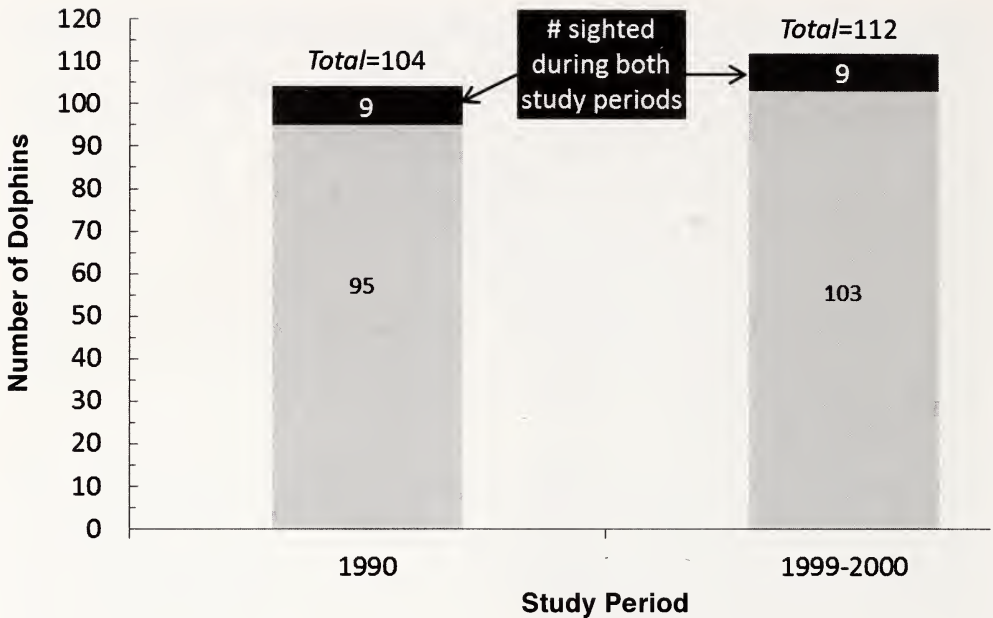


Fig. 3. Number of dolphins identified during the 1990 and the 1999–2000 San Quintín study periods. Some dolphins ( $n=9$ ) were sighted during both study periods, otherwise individuals were sighted only during the indicated study period.

### Results

During the 1990 and 1999–2000 study periods in San Quintín, 104 and 112 individuals were identified, respectively. Nine dolphins were identified in both the 1990 and 1999–2000 study periods while 95 of these individuals were sighted only during 1990 and 103 only in 1999–2000. The combined number of individuals identified in San Quintín during both study periods was 207 (Fig. 3). During the 1990 and 1999–2000 study periods, most individuals were sighted only one time; but some individuals were sighted on multiple occasions within a respective study period (Fig. 4).

Inter-study area match rates (MR) were derived by calculating the percent of individuals photographed in one study area, such as in San Quintín, that were also photographed in another study area. Similar match rate calculations were made for individuals photographed within the different CCSAs. The first comparison involved a composite of inter-study area matches reported for the two sampling periods when data were collected in the CCSAs: 1981–1989 (Defran et al. 1999) and 1996–2000 (Hwang et al. 2014) (Table 1). Match rates for the 1981–1989 sample were calculated by comparing the percent of dolphins identified in Ensenada ( $n=68$ , MR=88%), Orange County ( $n=133$ , MR=92%) and Santa Barbara ( $n=43$ , MR=88%) that matched to dolphins identified in San Diego ( $n=404$ ) where the sample size was highest. Match rates for the 1996–2000 sample were calculated by comparing the percent of dolphins identified in Ensenada ( $n=81$ , MR=43%) and Santa Barbara ( $n=182$ , MR=67%) that matched to San Diego ( $n=292$ ) where the sample size was again the highest. The combined 1981–1989 and 1996–2000 average match rate for the CCSAs was 76% ( $\pm 18.5$  S.D.).

The second comparison involved the inter-study area match rates between dolphins identified off San Quintín with dolphins in the combined 1981–1989 and 1996–2000 CCSAs catalog. Inter-study area matches occurred between both San Quintín datasets and

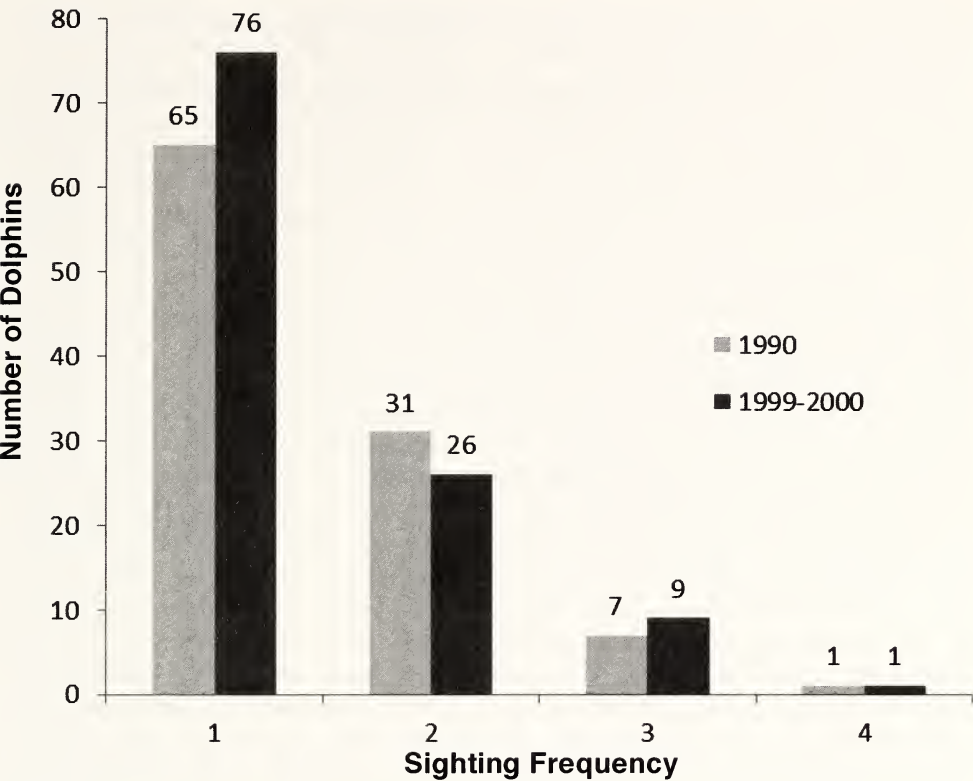


Fig. 4. Sighting frequency of dolphins during the 1990 and 1999–2000 San Quintín study periods.

the CCSAs catalog. In the 1990 San Quintín sample, 2 of the 104 dolphins identified were matched ( $MR=1.9\%$ ) to the combined CCSAs catalog. In the 1999–2000 San Quintín sample, 5 of the 112 dolphins identified were matched ( $MR=4.5\%$ ) to the combined CCSAs catalog. When dolphins identified off San Quintín in 1990 and 1999–2000 were combined ( $n=207$ ), 7 ( $MR=3.4\%$ ) were matched to dolphins in the CCSAs catalog.

Finally, 3 of the 7 dolphins matched between San Quintín and the CCSAs catalog were sighted in at least one of the CCSAs before and after their sighting(s) in San Quintín. The first of these dolphins was sighted before and during the 1990 San Quintín study period and again during the 1996–2000 CCSAs study period. The other two dolphins were sighted after the 1996–2000 CCSAs study period, during more recent surveys conducted in the San Diego study area between 2004–2011 (unpublished data, D. Weller). Of these seven matches, all were sighted in San Diego, two were also sighted in Ensenada, and two were also sighted in Orange County.

Discussion

Identifying population stock boundaries is important for management purposes in that it allows for a range-wide evaluation of potential threats. With such management considerations in mind, the most significant finding of this research was the low overlap ( $MR=3.4\%$ ) for dolphins photographed off San Quintín and those photographed in one or more of the CCSAs. In comparison, the overall match rate was considerably higher ( $MR=76\%$ ) between CCSAs study sites. These match rate differences suggest that both

a California coastal stock and Northern Baja California coastal stock exist, with only a limited degree of mixing between them.

Results from within the time frame of this research (i.e., 1990–1999) suggest that the northern range boundary for the proposed coastal Northern Baja stock is located somewhere between San Quintín and Ensenada. Although most individuals identified off San Quintín were sighted only once, the small number of individuals that were sighted multiple times within a given survey period provides some evidence for at least short-term use of the area. Similarly, the nine dolphins sighted during both San Quintín study periods indicate some degree of longer-term use of the area.

While the low match rate between San Quintín and the CCSAs suggests a small degree of overlap between the two proposed stocks, the total number of surveys conducted off San Quintín ( $n=20$ ) was relatively low in comparison to the number of surveys conducted in some of the CCSAs. That being noted, the number of surveys conducted off San Quintín ( $n=20$ ) is similar to the number of surveys off Ensenada ( $n=23$ ) that were also conducted during two distinct time periods that overlapped or nearly overlapped with the timing of the San Diego surveys. In this case, match rates for the Ensenada to San Diego photo-identification comparisons were markedly higher (MR=88% during 1981–1989; MR=43% during 1996–2000) than those found for the comparison of the CCSAs catalog with the two San Quintín survey periods (i.e., 1.9% and 4.5%, respectively). Further, while the number of San Quintín surveys was lower than those in the San Diego and Santa Barbara study areas, the number of dolphins identified was quite high. By way of comparison, the 207 individuals identified (sampled) in San Quintín was greater than the sample size in Ensenada (Table 1.,  $n=129$ ) and comparable to the total number of individuals in the Santa Barbara sample (Table 1.,  $n=213$ ). Thus, it is unlikely that the 3.4% inter-area match rate between San Quintín and the CCSAs is related to low survey effort or small sample sizes in San Quintín.

The primary variables contributing to the proposed stock structure are as yet unknown. Oceanographic and bathymetric variables have been hypothesized as potential habitat barriers for coastal bottlenose dolphins off California and Baja California (Caldwell, 1992) but verification of these mechanisms is unresolved. When stock separation occurs in bottlenose dolphins in the absence of confirmed geographic barriers, as is the case along the eastern North Pacific coastline (this research), as well as along the western North Atlantic Seaboard and within the northern inshore areas of the Gulf of Mexico (e.g., Texas, Florida), social structure, prey availability, and foraging specialization have been cited as possible foundations for dispersal tendencies (Sellas et al. 2005; Rosel et al. 2009; Toth et al. 2011). Such stock distinctions may be useful for management purposes, even when there is a moderate level of mixing with other adjacent stocks, such as that which occurs within Sarasota Bay and between nearby Gulf of Mexico inshore areas of Tampa Bay and Charlotte Harbor (see reviews in Selas et al. 2005; Rosel et al. 2009).

Complex social structure may act to minimize dispersal due to the investment required to build and maintain social bonds (Rosel et al. 2009). Social affiliations among California coastal dolphins, however, are highly dynamic (Weller 1991). Dispersal may also be limited in areas that have consistently high prey densities, allowing a population to be sustained long-term within a limited range. However, the regular travel of California coastal dolphins within their range suggests a patchy distribution of prey species requiring frequent relocation (Weller 1991; Hanson and Defran 1993; Defran et al. 1999; Ogle 2005; Hwang et al. 2014).

Among the inshore bottlenose dolphins found in some Atlantic Seaboard and Gulf of Mexico areas, as well as within Shark Bay, Australia, a number of foraging and resource specializations have developed. Over time, such specializations as strand feeding, sponge feeding and confinement to shallow water bays and estuaries for shark avoidance, could result in geographic range restrictions that give rise to stock separation (Silber and Fertl 1995; Connor et al. 2000; Sellas et al. 2005; Mann et al. 2008; Rosel et al. 2009). Similar mechanisms that might restrict the range of California coastal dolphins have not been observed.

Examination of the sighting records for the seven dolphins identified in both San Quintín and at least one of the CCSAs suggests that the mixing for some of these seven dolphins may not represent permanent immigration of California coastal dolphins into the putative coastal Northern Baja stock. Three of these seven dolphins were seen in at least one of the CCSAs both before and after their sightings in San Quintín. Thus, these dolphins appear to have visited San Quintín but subsequently returned to their putative range within the CCSAs. It is unknown whether such visits entail exploratory movements in search of prey and/or if they represent a mechanism by which some gene flow between the two stocks could be occurring. A final point relates to the 3.4% match rate reported in this research. The similarly low match rates observed for the two San Quintín sample periods (i.e., 1990=1.9%, 1999–2000=4.5%) suggests that the degree of mixing does fluctuate, at least to a small degree. Additional research conducted over years and varying oceanic conditions could provide a more sensitive measurement of dolphin mixing between the San Quintín and Southern California Bight study areas.

Thus far, the proposed stock separation presented herein relies entirely on photo-identification data. The differentiation of California coastal ecotype bottlenose dolphins from the offshore ecotype has successfully relied on the multiple data types and sources, including analyses of morphology, microbiology and genetics, as well as photo-identification (Walker 1981; Perrin et al. 2011; Bearzi et al. 2009; Lowther-Thieleking et al. 2014). Among these multiple data sources, genetic analyses have been particularly revealing in efforts to define and differentiate the coastal and offshore bottlenose dolphin stocks within California waters (Lowther-Thieleking et al. 2014), as well as in numerous other regions (Sellas et al. 2005; Rosel et al. 2009; Waring et al. 2012). Similar genetic data are needed, but remain to be collected from San Quintín coastal dolphins. Once such data are available, a genetic comparison to the California coastal stock can be made (Lowther-Thieleking et al. 2014). Combining genetic comparisons with future photo-identification data would provide a broader and more informed foundation from which management decisions can be made with regard to coastal bottlenose dolphins off California and Baja California.

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## Removal Efforts and Ecosystem Effects of Invasive Red Swamp Crayfish (*Procambarus clarkii*) in Topanga Creek, California

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**Abstract.**—Red swamp crayfish (*Procambarus clarkii*) were first recorded in Topanga Creek in 2001. When the onset of drought in Southern California resulted in low flows and warming water temperatures from 2011–2014, the population rapidly increased. Within the Santa Monica Mountains, *P. clarkii* has been linked to diminishing numbers of California newt (*Taricha torosa*), a species of special concern (Kats et al. 2013). To address these concerns, a student-based citizen science program was conducted from November 2013 through April 2014 to remove crayfish from a 200 m reach of Topanga Creek. The following data was collected and compared between the removal reach and an upstream, adjacent 200 meter non-removal reach (control): water quality (temperature, salinity, pH, conductivity, dissolved oxygen, turbidity), nutrient levels (nitrate, nitrite, ammonia, orthophosphate), benthic macroinvertebrate community metrics, crayfish demographics and catch-per unit effort (removal reach only). The results indicate that red swamp crayfish presence or removals do not affect water quality or nutrient levels in Topanga Creek. However, benthic macroinvertebrate communities were significantly different between reaches; the presence of crayfish correlated with lower BMI abundance and species richness, higher proportion of tolerant taxa, and lower feeding group complexity.

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Red swamp crayfish (*Procambarus clarkii*) have spread far across the globe, posing an invasive threat to freshwater species abundance and community diversity (Ficetola et al. 2011). Mediterranean wetlands, such as those found along the southern coast of California, have been shown to be preferred habitat for *P. clarkii* in periods of drought with reduced flows and increased water temperatures (Geiger et al. 2005). This crustacean grows rapidly, maturing within three months after hatching, and can reproduce twice a year in warm conditions (Barnes 1974; Vodopich and Moore 1999). Large healthy females typically produce 600 viable young furthering their ability to spread quickly (Barnes 1974; Vodopich and Moore 1999). *Procambarus clarkii* are omnivorous consumers of an array of plant and animal matter such as macrophytes, detritus, amphibian eggs and larvae, aquatic invertebrates, and small fish, thus affecting the riparian food web on a polytrophic scale (Momot et al. 1978; Momot 1995; Stenroth and Nyström 2003). The generalist and predatory feeding habits of *P. clarkii* have been linked to observed declines in macrophyte abundance (Feminella and Resh 2006; Rodriguez et al. 2005), amphibian species richness and recruitment (Gamradt and Kats 2002; Cruz et al. 2006; Ficetola et al. 2011), and macroinvertebrate diversity (Correia and Anastácio 2008).

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Red swamp crayfish were detected in southern California as early as 1924 (Holmes 1924), but not observed in Topanga Creek until 2001 (*RCDSMM unpublished data*). Topanga Creek is the third largest coastal watershed (47 km<sup>2</sup>) draining into the Santa Monica Bay. Freshwater systems in this region are critical habitat that support a number of sensitive and endangered native aquatic species. *Procambarus clarkii* were the first introduced fauna to become established and spread throughout Topanga Creek, and remains the most abundant non-native invasive in the watershed. The population of *P. clarkii* in Topanga Creek was initially suppressed by active removal efforts and significant winter rain events and sufficient flows to reduce crayfish abundance (Kats et al. 2013). Below average rainfall and low flows in 2011–2014 have facilitated the extensive establishment of *P. clarkii* throughout Topanga Creek.

The population growth of *P. clarkii* in Topanga Creek raised concerns about possible implications for two native species, the California newt (*Taricha torosa*), a California species of special concern, and federally endangered southern steelhead trout (*Oncorhynchus mykiss*). Data collected from Topanga Creek during snorkel and other visual surveys (2001–2014) documented the spread and increased abundance of *P. clarkii*, as well as provided direct observations of crayfish attacking newts (*RCDSMM unpublished data 2014*). The interactions of crayfish and *O. mykiss* are less clear; however, since 2011 an increased incidence of crayfish found in the diet of large (>25.4 cm) *O. mykiss* has been observed (Krug et al. 2012).

Benthic macroinvertebrates (BMI) are an important food source for both *P. clarkii* and *O. mykiss* (Angradi and Griffith 1990, Nystrom and Graneli 1996). Competition for food resources and disruption of BMI community functionality is a potential concern. The complexity of functional feeding groups (e.g., gatherers, filterers, scrapers, predators) can be a measure of the functional integrity of BMI communities and a reflection of its capacity to cycle nutrients (Wallace and Webster 1996). Disturbance to the benthic community, such as the introduction of non-native fauna, can alter BMI community composition and cause unanticipated changes in freshwater ecosystems (Covich et al. 1999). Changes in BMI abundance, diversity, and feeding group complexity can indicate such community disturbance.

In Topanga Creek, drought induced low flows in 2011–2014 resulted in isolated refugia pools and reduced numbers of *O. mykiss* redds and young of the year<sup>1</sup>. However, *P. clarkii* were able to successfully reproduce and inhabit the shallow riffles and fragmented reaches inaccessible to *O. mykiss*. In September 2013, the Resource Conservation District of the Santa Monica Mountains (RCDSMM), in conjunction with the Watershed Stewards Program (WSP), launched a citizen science program that 1) removed crayfish from several refugia step-pool habitats within a 200 meter reach of Topanga Creek, 2) measured crayfish demographics (sex/length), and 3) monitored water quality (dissolved oxygen, pH, salinity, conductivity, turbidity, water temperature), nutrient levels (nitrate, nitrite, ammonia, phosphate), and BMI community metrics.

### Materials and Methods

Topanga Creek (34° 6'11"N 118° 36'18" W, gradient 1 to 6%) is the main drainage of a small coastal watershed (approximately 47 km<sup>2</sup>) located within the Santa Monica

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<sup>1</sup> Krug, J., R. Dagit, Stillwater Sciences, and J.C. Garza. 2014. Lifecycle monitoring of *Oncorhynchus mykiss* in Topanga Creek, California. Final Report Prepared for CA Department of Fish and Wildlife, Contract No. PO950013. January 2014.

Mountains National Recreation Area in southern California. The study reach consisted of 400 continuous meters in Topanga Creek, starting at 3500 m (upstream from the ocean) and ending at 3900 m. The study area included a downstream 200 m crayfish removal reach (RR), and an upper 200 m non-removal reach (NRR; Fig. 1). Both reaches were relatively uniform in geomorphological features, including a similar distribution of pools, step-pools, runs, and riffles, substrate type, and percent canopy cover. No introduced barriers of any sort were incorporated into the study reaches; however, natural low-flow boulder barriers separated the RR from the NRR.

A total of ten volunteer crayfish removal events took place between September 2013 and April 2014. Water quality, nutrient, and BMI samples were collected in both 200 m reaches during removal events between November 2013 and April 2014. Crayfish were removed throughout RR with 7.6 cm hot dog pieces attached to hemp strings. The presence of federally listed *O. mykiss* prevented setting traps of any kind. Crayfish were counted, sexed, and measured (cm) from the tip of the rostrum to the end of the tail in midline. Removed crayfish were donated to a local wildlife rescue or used for educational purposes.

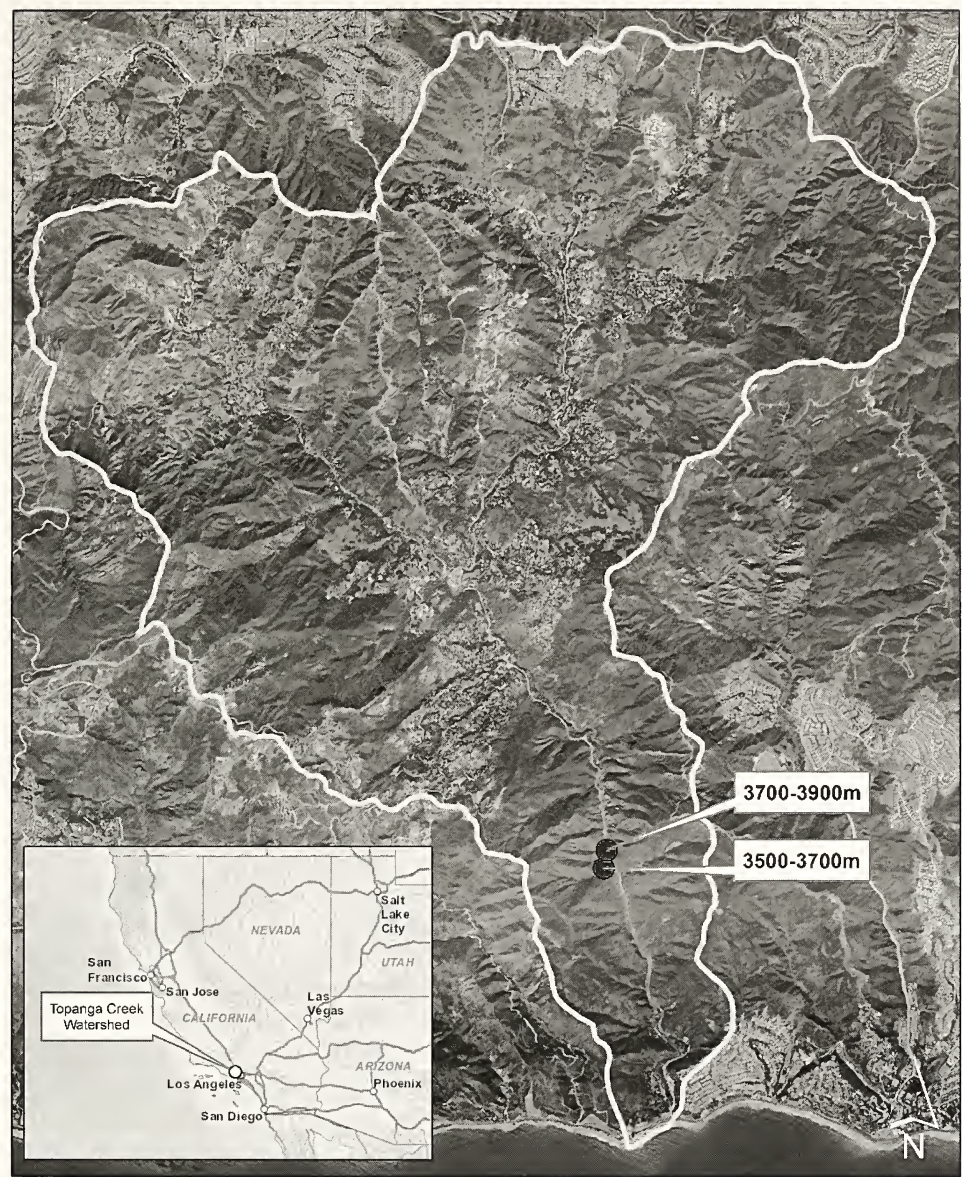
Water samples were collected from three pools within each 200 m reach an hour prior to removal. Each site was tested for air temperature (mercury thermometer), salinity (ATC 300011 SPER SCIENTIFIC salt refractometer), pH (Oakton pHTestr 30), conductivity (Oakton ECTestr11), dissolved oxygen (DO) and water temperature (YSI 55 DO meter). All probes were calibrated within a week prior to the collection date. Nutrient and turbidity sampling was conducted once a month from November 2013 through April 2014 at 3500 m, 3550 m, and 3600 m in RR and at 3700 m, 3800 m, and 3850 m in NRR. Samples were tested for nitrate-N (ppm), nitrite-N (ppm), ammonia-N (ppm), orthophosphate (ppm) and turbidity (NTU) within eight hours of collection using LaMotte SMART3 colorimeter and LaMotte 2020we turbidity meter.

BMI samples were collected according to CA Rapid Bioassessment protocol<sup>2</sup> in November 2013, December 2013, February 2014, and April 2014 at three comparable sites in RR and NRR. Each sample was composed of nine kicks into a 1-ft. wide D-frame net (three transects and three kicks per transect). Samples were preserved in 95% ethanol and processed within a month from collection date. BMI were identified to genus, or lowest possible taxonomic level using a 40x magnification dissecting microscope. *P. clarkii* was recorded but not included as a benthic macroinvertebrate for analysis. For quality assurance, 10 percent of samples were randomly selected and re-identified by a second processor. First and second identifications were compared and scored for accuracy, resulting in an estimated error of 1.6%.

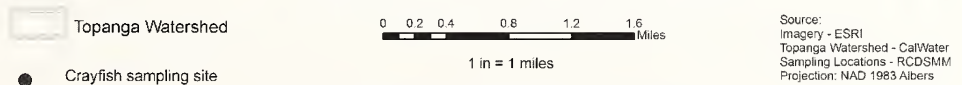
Paired t-tests were applied to determine any significant difference between the two reaches in crayfish demographics, water quality, nutrient levels, and biotic integrity metrics of BMI communities. Regression analyses were performed to compare water quality metrics to crayfish removal and to analyze the relationship between catch per unit effort and water temperature. Simpson's Index of Diversity (Simpson 1949) was calculated for each BMI sample and analyzed by paired t-test to compare biodiversity. Simpson's was also applied to samples categorized by functional feeding groups (gatherers/filterers, scrapers, predators, or other) to compare feeding group complexity. Southern Coastal California Index of Biotic Integrity (SCC-IBI; Ode et al. 2005) metrics

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<sup>2</sup>Ode, P.R. 2003. CAMLnet: list of California macroinvertebrate taxa and standard taxonomic effort. Aquatic Bioassessment Laboratory, Rancho Cordova. Retrieved September 10, 2014 from <http://www.safit.org/ste.html>.



Topanga Creek Sampling Locations



\*\*Distances are linear meters from the ocean

Fig. 1. Map of Topanga Creek Watershed and the crayfish study reaches (3500–3700 Removal Reach (RR); 3700–3900 Non-Removal Reach (NRR)).

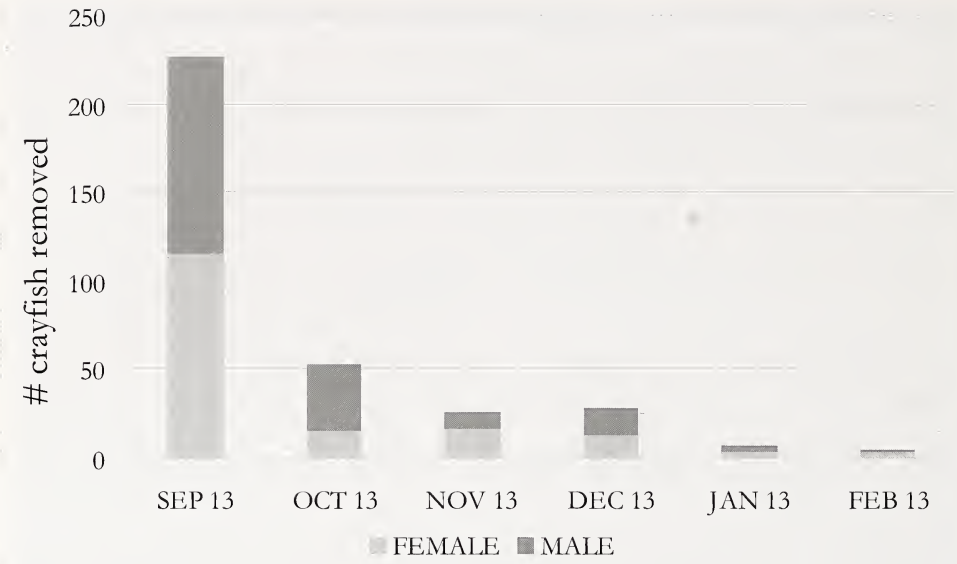


Fig. 2. Total number of male and female crayfish removed each month Oct. 2013 to Feb. 2014.

(number of EPT, Coleoptera and predator taxa, and percent tolerant, intolerant, non-insect, and collector-gatherers + collector-filterers) were applied and scored for all BMI samples.

### Results

Ten volunteer removal events between September 2013 and April 2014 (203.25 person-hours) resulted in the removal of 345 *P. clarkii*; 166 females and 179 males (Fig. 2). The average length of crayfish removed was  $7.61(\pm 0.348 \text{ SE})$  cm. There was no significant difference between male and female average length or number removed. The first event (9/21/2013) resulted in the most captures with more than four times as many crayfish removed than any proceeding month. The catch per unit effort (CPUE) in the study period November 2013 to February 2014 ranged from 0.1 to 3.0 crayfish per person per hour, and increased significantly with warmer water temperatures ( $R^2=0.67$ ,  $F=12.27$ ,  $p<0.05$ ). An increase of approximately 0.26 CPUE was calculated for every  $1^\circ\text{C}$  increase in temperature (Fig. 3). The comparison of water quality and nutrient concentrations between the RR and NRR showed no significant differences, except for salinity. Salinity showed a statistical difference between reaches (paired two-tailed,  $t(3)=-4.65$ ,  $p<0.02$ ). The NRR had higher salinity throughout the course of the study, although levels in both reaches ranged from 0–2 ppm.

The four BMI samples collected from the NRR in November 2013, December 2013, February 2014, and April 2014 contained a total of 645 individuals and 38 taxa. The samples collected from the RR contained a total of 3,642 individuals and 51 taxa. A total of four phyla were represented including Arthropoda, Annelida, Mollusca, and Nematoda. BMI abundance was significantly higher (paired two-tailed  $t(3)=3.59$ ,  $p<0.04$ ) in the RR (Fig.4). In both reaches, there was an increase in BMI abundance from November through April. The NRR had significantly lower richness (paired one-tailed  $t(3)=2.74$ ,  $p<0.04$ ). However, species diversity as measured by Simpson's Index of

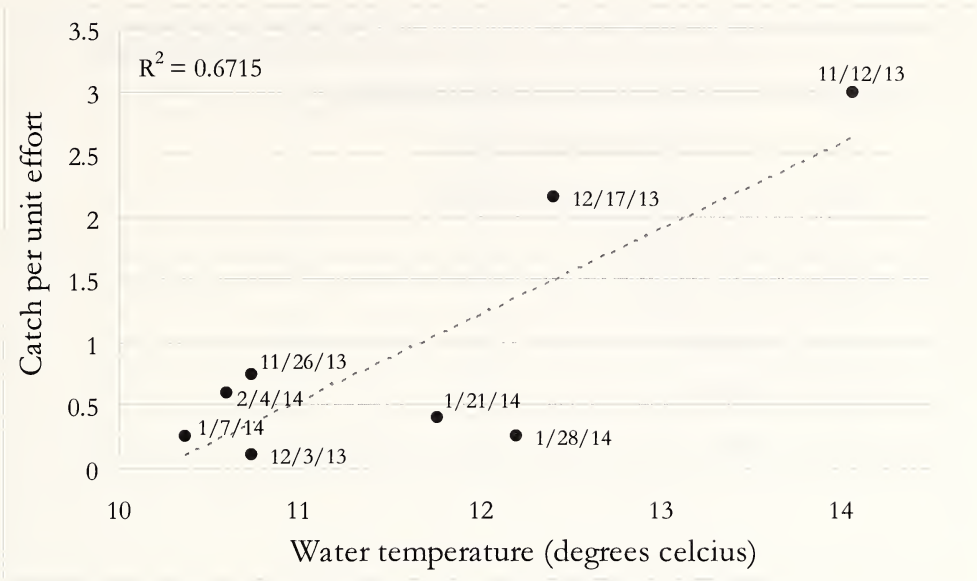


Fig. 3. Relationship between CPUE (catch/person/hour) and Water Temperature (°C) in Topanga Creek Nov. 2013 to Feb. 2014.

Diversity (Simpson 1949) was not significantly different between sites and ranged from 0.66 to 0.84 for all samples.

In the RR, the three most dominant taxa were Chironomidae (midge larvae, 24%), freshwater snails (Viviparidae and Hydrobiidae, 22% relative abundance), and *Hyaella* (freshwater Amphipod, 15%) (Fig. 5). The three most abundant taxa in the NRR were

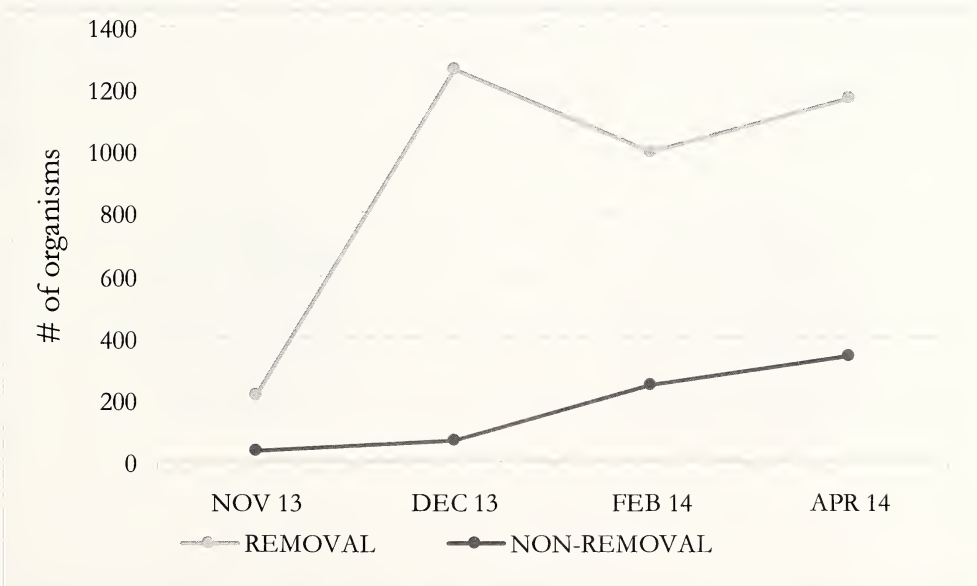


Fig. 4. Benthic macroinvertebrate abundance in samples collected from removal (RR) and non-removal (NRR) Nov. 2013 to Apr. 2014.

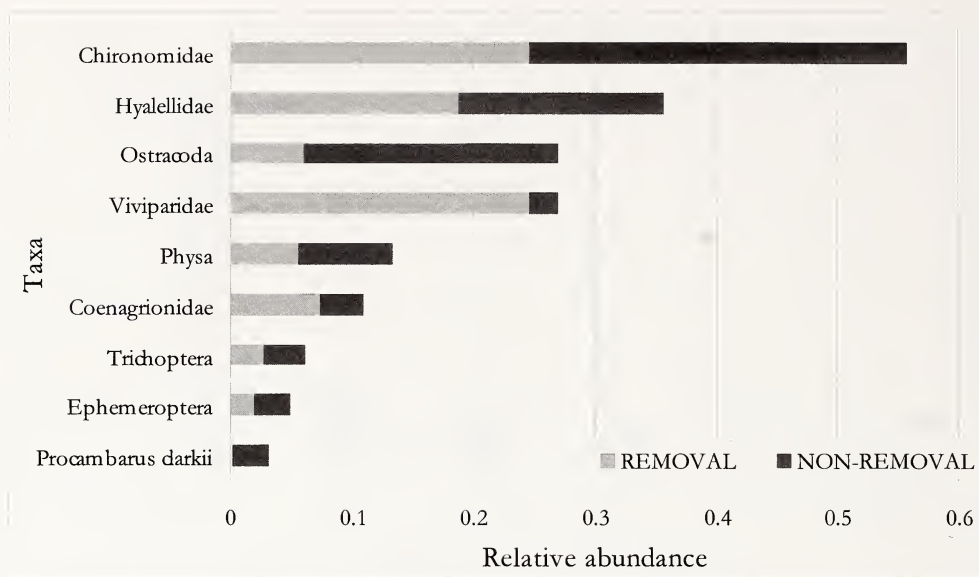


Fig. 5. Eight dominant taxa collected in each 200m study reach.

Chironomidae larva (33%), Ostracoda (seed shrimp, 22%), and *Hyalella* (18%). The two reaches shared the same six most dominant species, including the above mentioned with the addition of Coenagrionidae (narrow-winged damselfly nymphs) and *Physa* (physa snails). These dominant taxa described above each have a tolerance value of 8, with the exception of Chironomidae, which has an assigned tolerance value of 6 although there is great variation among genera and species.

While total SCC-IBI scores showed no trend, two SCC-IBI metrics differed significantly between sites: % tolerant taxa and % collector-gatherer + collector-filterer. The NRR had greater % tolerant taxa (tolerance values 8-10) than RR (paired two-tail  $t(3) = -5.24$ ,  $p < 0.02$ ). The NRR had a greater proportion of collector-gatherer and collector-filterer organisms (paired two-tail  $t(3) = -3.70$ ,  $p < 0.04$ ) and fewer scraper organisms (paired two-tail

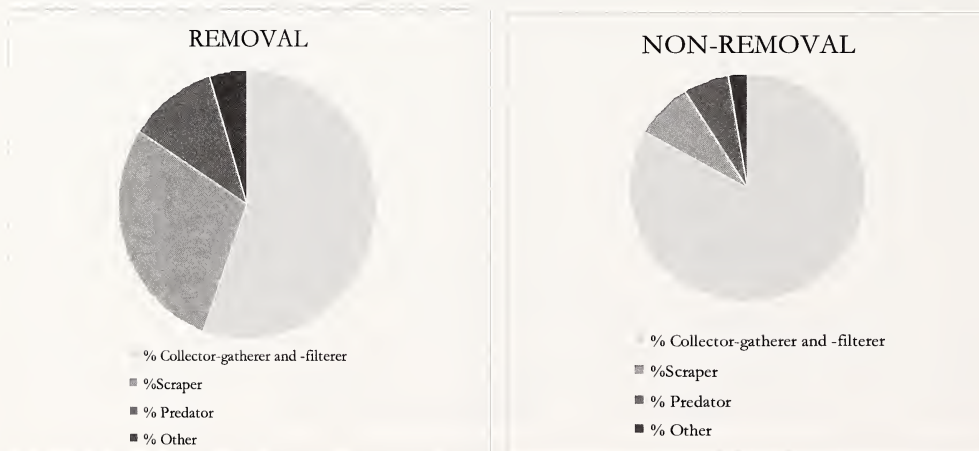


Fig. 6. Average functional feeding group composition in removal and non-removal reach samples Nov. 2013 to Apr. 2014.

$t(3)=4.05$ ,  $p<0.03$ ). In applying Simpson's Index to sample data categorized by functional feeding groups, functional feeding group diversity was significantly higher in the RR (two-tailed,  $t(3)=3.41$ ,  $p<0.05$ ) (Fig. 6). Additionally, *P. clarkii* were collected more often in NRR BMI samples (3.1%, 20 individuals total) than in RR (<1%, 7 ind.).

### Discussion

The invasive *Procambarus clarkii* has been shown to have severe effects on native aquatic wildlife in southern California streams (Riley et al. 2000, Gamradt and Kats 2002, Rodriguez et al. 2005, Cruz et al. 2006, Feminella and Resh 2006, Correia and Anastácio 2008, Ficetola et al. 2011). In Topanga Creek, benthic macroinvertebrate abundance and species richness were significantly higher in the 200m RR where crayfish were actively managed by hand-removal than in an adjacent NRR. This result is consistent with previous reports that correlate non-native crayfish presence to reduced BMI abundance in freshwater systems (Charlebois and Lamberti 1996, Stewart et al. 1998). In the RR, BMI samples contained between 23 and 51 distinct taxa and in the NRR, richness ranged from 6-38. This finding corroborates previous studies that have found that *P. clarkii* invasions lead to loss of BMI diversity (Rodriguez et al. 2005, Correia and Anastácio 2008). Functional feeding group diversity was lower in the NRR, and % of tolerant organisms was higher.

Increased abundance of BMI in RR indicates higher productivity for a number of taxa. Six distinct taxa had more than 100 individuals in one or more samples from the RR including Viviparidae and Hydrobiidae, Chironomidae, Hyalellidae, Coenagrionidae, Ostracoda, and Physa. Only two taxa had more than 100 individuals in any one NRR sample: Chironomidae and Ostracoda. A major distinction between community was that Viviparidae and Hydrobiidae were most abundant taxa in RR, but relatively rare in NRR (3%). The relative rarity of freshwater snails (scrapers) in the NRR diminished feeding group complexity. *Procambarus clarkii* predation on Viviparidae in this reach is one possible driver of reduced abundance of the genus, although micro-habitat differences within the 400 m study reach are another potential factor. Higher abundance, species richness, feeding group complexity, and a smaller proportion of tolerant species indicate that the BMI community in RR was in better ecological condition than in NRR. As crayfish are generally the largest species within the BMI community, a comparison of BMI sample proportional dry weight of taxa groups would further our understanding of *P. clarkii* effects on trophic-level productivity by providing a quantitative measure of biomass.

The ecological implications of invasive *P. clarkii* in Topanga Creek could be severe if they significantly disrupt benthic macroinvertebrate communities. BMI make up the primary consumer trophic level and play an integral part in nutrient decomposition and cycling through riparian systems. Changes at this level could impact higher trophic organisms such as California newts (species of special concern) and southern California steelhead trout (endangered). How the continuation of drought conditions within the region will continue to affect the population and impact of *P. clarkii* is uncertain; reduced flows and higher temperatures place stress upon aquatic natives, it renders riparian habitat more preferential for crayfish.

Water quality and nutrient results between reaches were less notable. Salinity was the only parameter to differ significantly, which may be influenced by a groundwater seep in NRR at 3900 m<sup>3</sup>. Some studies have suggested *P. clarkii* may be a source of bioturbation

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<sup>3</sup> GeoPentech. 2006. Hydrogeologic Study Lower Topanga Creek Watershed, Los Angeles County, CA. Prepared for the RCD of the Santa Monica Mountains. Topanga, CA.

(Mueller 2007, Yamamoto 2010), however, results in this study showed no significant difference in turbidity between the RR and the NRR.

The level of effort per crayfish removed increased over the course of the study at a rate that correlated to decreasing water temperatures. While decreased activity is one possible factor, diminished crayfish numbers due to removal efforts is another. Removal events might be most efficient in warmer months; however a more extensive study including more removal areas and a longer time period is needed to determine whether there is a relationship between temperature and catch per unit effort, as well as to more completely characterize the effects of crayfish on water quality and the benthic macroinvertebrate community in Topanga Creek.

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## **Soil Organic Carbon and Nitrogen Storage in Two Southern California Salt Marshes: The Role of Pre-Restoration Vegetation**

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**Abstract.**—Soil organic carbon and nitrogen storage represent important ecosystem services provided by salt marshes. To test the importance of vegetation on soil properties, we measured organic carbon, total nitrogen, and belowground biomass in two southern California salt marshes. In both marshes, cores were collected from areas which differed in dominant vegetation cover prior to the restoration of tidal influence. There were no differences in organic carbon or total nitrogen density between vegetation classes at either site; however, a relationship between belowground biomass and soil organic carbon suggests that vegetation may influence soil properties.

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Salt marshes provide a number of important ecosystem services, including habitat for fish and bird species, food web support for adjacent marine environments, nutrient removal from the landscape, and carbon storage in long-lived soil pools (e.g., Zedler and Kercher 2005). Despite their importance, these ecosystems have been lost at alarming rates. Recent estimates suggest that on a global scale, 25% of salt marshes have been lost since the 1800s with ongoing loss rates of an additional 1–2% per year (McLeod et al. 2011). While comparable estimates of loss rates in southern California are limited, it is likely that salt marsh loss in the region is considerably higher than the global average. Grossinger et al.<sup>1</sup> used US Coast Survey T-sheets from the late 1800s to estimate a historical area of 7,711 ha of vegetated intertidal marsh along the South Coast of California (from Point Conception to the Mexico border). Sutula et al.<sup>2</sup> estimated that approximately 1681 ha (4,153 acres) of intertidal estuarine wetlands remain in the same region. While a direct comparison between these values should be viewed with caution due to differences in methodologies, the apparent dramatic loss in wetland area highlights the impact of historical anthropogenic activities on Southern California wetlands. More recently, losses of salt marsh habitat in the Pacific region were negligible between 2004–2009 (Dahl and Stedman 2013), suggesting that rates of loss have slowed. Further, ongoing conservation and restoration activities are aimed at maintaining the services provided by the remaining wetlands in the region (Callaway and Zedler 2009).

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<sup>1</sup> Grossinger, R.M., E.D. Stein, K.N. Cayce, R.A. Askevold, S. Dark and A.A. Whipple. 2011. Historical wetlands of the southern California coast: an atlas of US Coast Survey T-sheets, 1851-1889. San Francisco Estuary Institute Contribution #586 and Southern California Coastal Water Research Project Technical Report #589, 55 pp.

<sup>2</sup> Sutula, M., J.N. Collins, A. Wiskind, C. Roberts, C. Solek, S. Pearce, R. Clark, A.E. Fetscher, C. Grosso, K. O'Connor, A. Robinson, C. Clark, K. Rey, S. Mrrissette, A. Eicher, R. Pasquinelli, M. May and K. Ritter. 2008. Status of Perennial Estuarine Wetlands in the State of California. Southern California Coastal Water Research Project, 48 pp.

A great deal of recent attention has focused on capitalizing on ecosystem services provided by salt marshes as a potential means to support ongoing restoration and conservation efforts. In particular, there is a growing interest in quantifying carbon storage in salt marshes (Chmura et al. 2003; Mcleod et al. 2011; Pendleton et al. 2012). Salt marshes, along with other vegetated coastal ecosystems including mangroves and sea grass beds, are particularly effective at storing carbon in their soils because anaerobic conditions generally limit decomposition of primary productivity in these ecosystems (Meronigal et al. 2004; Tobias and Neubauer 2009) while a continuous supply of sulfate limits production of the greenhouse gas methane (Poffenbarger et al. 2011). Further, salt marshes continuously accrete new soils vertically to cope with sea level rise, which allows for new layers of soil carbon to be accumulated through time (Kirwan and Meronigal 2013; Morris et al. 2002). This so-called “blue carbon” could conceptually be traded in emerging carbon markets, although there are a number of ecological, political and economic questions surrounding this possibility (Edwards et al. 2013; Pendleton et al. 2013; Sutton-Grier et al. 2014; Ullman et al. 2013). Concomitant with storing “blue carbon”, salt marsh soils serve as an important sink for nitrogen, and this ecosystem service may also be valuable in the context of restoration and conservation efforts (Lau 2013).

We have previously measured soil organic carbon storage in two restored salt marshes in Huntington Beach, California (Keller et al. 2012). This work showed that soil organic carbon was generally higher in a marsh that had been restored for two years than in an adjacent marsh that had been restored for 22 years. This suggests that the assumption that restoration projects share a common starting point and predictably accumulate soil carbon through time needs to be critically evaluated. In particular, we hypothesized that initial site conditions, such as extant vegetation, may be as important as time following restoration when determining soil carbon storage, and perhaps when determining other belowground ecosystem properties.

Here, we further explore this possibility by measuring soil carbon and nitrogen storage, as well as belowground biomass, in two additional southern California salt marshes. In the first marsh, which had been restored for three years, we compared belowground properties from areas which differed in vegetation coverage prior to restoration. In the second marsh, which had not yet been restored, we compared areas dominated by dramatically different pre-restoration vegetation communities.

## Materials and Methods

### *Site Description*

The Huntington Beach Wetlands used for this project are remnants of a larger marsh that historically existed at the mouth of the Santa Ana River in northern Orange County, California (33° 39' N, 117° 59' W). The majority of this marsh area was isolated from tidal exchange by the mid-1940s due to development and flood control measures, but various wetland restoration efforts, including reconnection to tidal exchange, have been taking place since the 1980s<sup>3</sup>. To explore the importance of pre-restoration vegetation on belowground carbon and nitrogen dynamics, we collected samples in both the Magnolia and Newland Marshes (Fig. 1).

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<sup>3</sup> Jones & Stokes Associates, I. 1997. Talbert Marsh restoration project five-year postrestoration monitoring report. Final. December. (JSA 96-300.) Sacramento, CA. Prepared for Huntington Beach Wetlands Conservancy, Huntington Beach, CA.

Efforts to restore the 16.6 ha Magnolia Marsh, including reestablishment of tidal influence as well as the recreation of historical tidal channels, were completed in 2010 (Gordon Smith, Huntington Beach Wetlands Conservancy, personal communication). We utilized Google Earth images from October of 2007 to identify locations which differed in pre-restoration vegetation coverage. Specifically, we collected four soil cores from areas with vegetation cover prior to restoration (“vegetated”) and two soil cores from areas with limited vegetation cover (“unvegetated”; Fig. 1C.). While admittedly qualitative, our designations of vegetation cover are in general agreement with vegetation monitoring efforts at Magnolia Marsh, which show extensive coverage of senescent salt marsh vegetation on the eastern side and limited vegetation on the western side of this site<sup>4</sup>. Core locations were not selected based on specific vegetation communities, but at the time of collection vegetation was generally similar to other southern California salt marshes, and included: pickleweed (*Salicornia pacifica*), alkali seaheath (*Frankenia salina*), turtleweed (*Batis maritima*), and saltgrass (*Distichlis spicata*).

At the time of our sampling, tidal influence had not yet been restored to the 17.8 ha Newland Marsh, located west of Magnolia Marsh in Huntington Beach. This site currently has two visually distinct vegetation communities; a salt marsh community (“salt marsh”) dominated by plants similar to those found in Magnolia Marsh and a brackish community (“brackish”) dominated by cattail (*Typha* sp.) We collected two soil cores from each vegetation community in Newland Marsh (Fig. 1D.).

### *Sample Collection and Analysis*

Soil cores were collected in October-December 2013 following a modification of the protocol described in Keller et al. (2012). Briefly, a 15.3 cm diameter stainless steel tube equipped with a sharpened bottom edge was inserted to an average depth of 41 cm below the soil surface (range 32–48 cm). Care was taken to minimize soil compaction. Upon extraction of the core, soils were sliced into 2 cm depth increments using a serrated knife and returned to the laboratory at Chapman University for processing. Each depth increment was weighed and then passed through a 2 mm sieve within 1 week of collection (when necessary, soils were stored at 4°C until sieving). Material >2mm was subsequently washed with distilled water over a 1-mm sieve and live roots and rhizomes were collected and dried at 60°C to a constant mass. Four depths from a core collected in the brackish community at Newland Marsh had highly organic soils, which did not pass easily through the 2 mm sieve. Belowground biomass was removed by hand from these depths and the remaining (unsieved) soil was processed as described below. Subsamples of soil that passed through the 2 mm sieve were dried at 60°C to determine percent moisture for each depth increment. Percent moisture values were used to calculate the total dry mass of soil based on the total wet mass collected at each depth. Dried soils were ground to a fine powder using an IKA A11 Basic Analytical Mill (IKA Works, Inc., Wilmington, NC, USA). Organic carbon and total nitrogen were measured using a Costech elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA). To remove inorganic carbon, soil samples were acidified with 50 µL of 1M HCl and dried overnight at 37°C twice before carbon and nitrogen analysis (Craft et al. 1991).

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<sup>4</sup>Whitcraft, C., B. Allen and C. Lowe. 2013. Huntington Beach Wetlands Restoration Project Monitoring Program Methodology and Data Summary. Prepared for Huntington Beach Wetlands Conservancy and National Oceanic and Atmospheric Administration – MSRP.

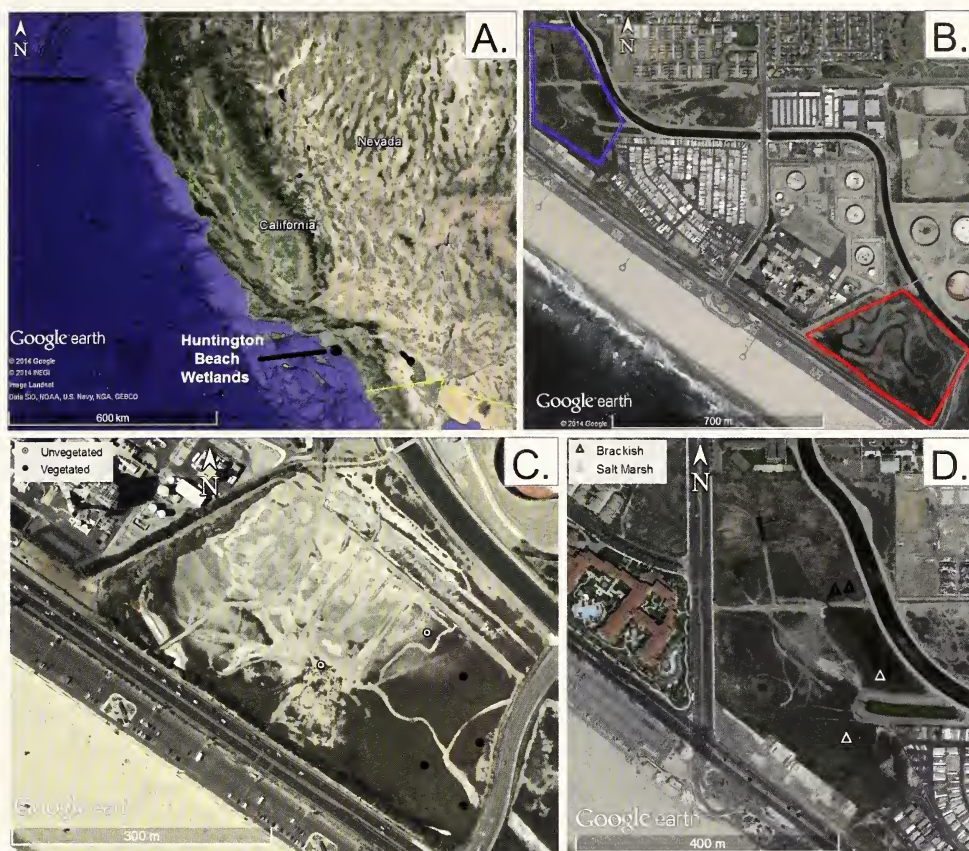


Fig. 1. Location of Huntington Beach Wetlands (A.) and of Magnolia Marsh (outlined in red) and Newland Marsh (outlined in blue) (B.). Soil cores were collected from areas identified as vegetated ( $n=4$ ) or unvegetated ( $n=2$ ) prior to restoration in Magnolia Marsh based on imagery from 2007 (image date: 10/22/2007) (C.). Soil cores were collected from areas dominated by brackish ( $n=2$ ) or salt marsh ( $n=2$ ) vegetation in Newland Marsh (image date: 4/16/2013) (D.). Image source: Google Earth for all panels.

Organic matter content was determined as loss on ignition (LOI) following combustion at  $400^{\circ}\text{C}$  for at least 10 hours.

### Statistical Analyses

Organic carbon and total nitrogen concentrations were multiplied by the total dry mass of soil to calculate the mass of organic carbon and total nitrogen in each depth increment. These values were subsequently summed over the 0–10 and 0–40 cm depth increments and expressed as organic carbon or total nitrogen densities ( $\text{g cm}^{-3}$ ) based on the total volume of these depth ranges (Keller et al. 2012). The 0–10 cm depth increment includes the majority of roots found in these sites while the 0–40 cm depth increment includes the entirety of the soil core. In cases where soils cores did not extend to a depth of 40 cm, the average elemental and mass values from the 3 deepest depth increments were used for all missing depths to 40 cm. In 2 cores from Newland Marsh, this approach was used for the 38–40 cm depth increment. In a single unvegetated core from Magnolia Marsh, averages were used for the 32–40 cm depth increments. A similar approach was used to calculate total belowground biomass (g) in the upper 10 and 40 cm of each soil core.

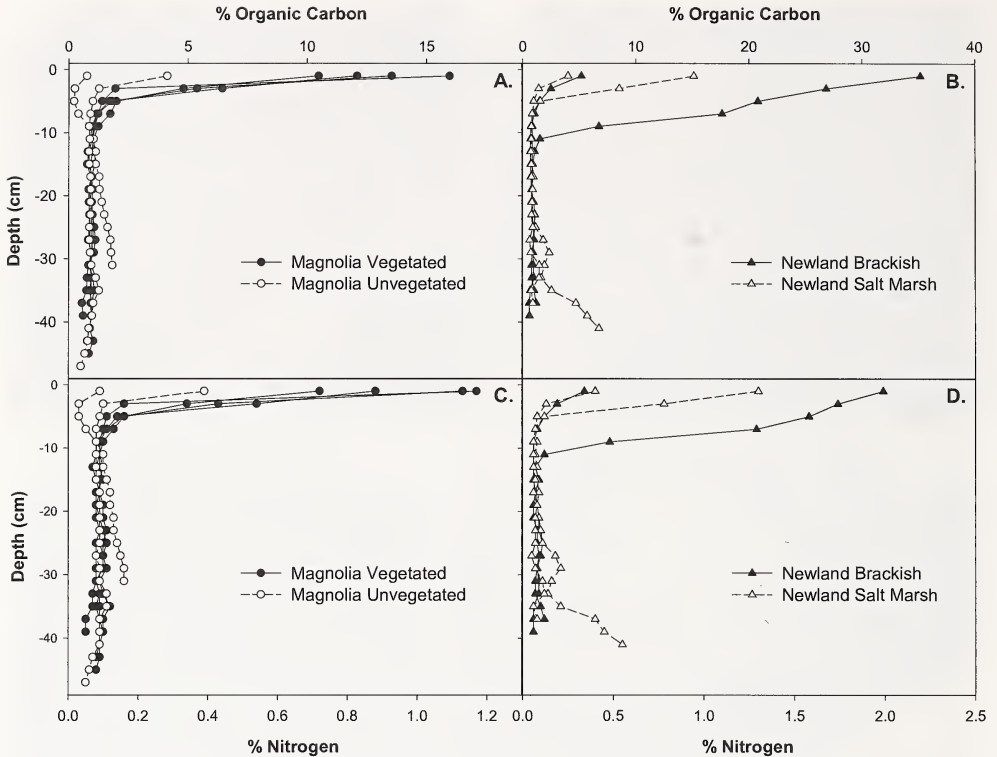


Fig. 2. Depth profiles of soil organic carbon content (A., B.) and total nitrogen content (C., D.) in cores collected from Magnolia and Newland Marshes. In Magnolia Marsh, cores were collected from areas identified as vegetated ( $n=4$ ) or unvegetated ( $n=2$ ) prior to restoration. In Newland Marsh, cores were collected from areas dominated by Brackish ( $n=2$ ) or Salt Marsh ( $n=2$ ) vegetation.

Independent t-tests were used to compare organic carbon densities, total nitrogen densities and belowground biomass in the 0–10 and 0–40 cm depth increments between vegetated and unvegetated cores in Magnolia Marsh and between brackish and salt marsh cores in Newland Marsh. All data were normally distributed; however, data frequently failed to meet assumptions of equal variance between groups based on the Levene's Test. In cases with unequal variances, we used the more conservative t-test output that did not make assumptions about equal variance (IBM Corp 2012). Differences were considered significant at  $p < 0.05$  for all t-tests. Regressions were used to explore relationships between LOI, organic carbon and total nitrogen content as well as relationships between soil organic carbon density and belowground biomass. All analyses were completed using Version 21 of the IBM SPSS statistical package (IBM Corp 2012).

### Results

Organic carbon content was highest in surface soils and decreased with depth at both Magnolia Marsh and Newland Marsh (Fig. 2A and B.). Vegetated cores at Magnolia Marsh had higher average organic carbon concentrations than unvegetated cores in the upper 10 cm, but these differences disappeared at deeper depths (Fig. 2A.). Average carbon density to a depth of 10 cm in vegetated cores at Magnolia Marsh was nearly double the carbon density in unvegetated cores; however, there were no significant differences in carbon density between vegetated and unvegetated cores over either the

Table 1. Mean ( $\pm 1$  SE) soil organic carbon density, total nitrogen density and belowground biomass in soil cores collected from Magnolia and Newland Marshes. All values were summed to a depth of either 10 cm or 40 cm. There were no significant differences between vegetated and unvegetated samples in Magnolia Marsh or brackish and salt marsh samples in Newland Marsh at either depth.

|                                               | Magnolia Marsh       |                      | Newland Marsh        |                      |
|-----------------------------------------------|----------------------|----------------------|----------------------|----------------------|
|                                               | Vegetated<br>(n=4)   | Unvegetated<br>(n=2) | Brackish<br>(n=2)    | Salt Marsh<br>(n=2)  |
| Organic Carbon Density ( $\text{g cm}^{-3}$ ) |                      |                      |                      |                      |
| 0–10 cm                                       | 0.023 $\pm$ 0.0014   | 0.012 $\pm$ 0.0045   | 0.026 $\pm$ 0.0092   | 0.022 $\pm$ 0.0044   |
| 0–40 cm                                       | 0.013 $\pm$ 0.0002   | 0.013 $\pm$ 0.0024   | 0.014 $\pm$ 0.0018   | 0.016 $\pm$ 0.0016   |
| Total Nitrogen Density ( $\text{g cm}^{-3}$ ) |                      |                      |                      |                      |
| 0–10 cm                                       | 0.0019 $\pm$ 0.00013 | 0.0012 $\pm$ 0.00003 | 0.0018 $\pm$ 0.00060 | 0.0020 $\pm$ 0.00030 |
| 0–40 cm                                       | 0.0012 $\pm$ 0.00030 | 0.0012 $\pm$ 0.00020 | 0.0012 $\pm$ 0.00005 | 0.0014 $\pm$ 0.00015 |
| Belowground Biomass (g)                       |                      |                      |                      |                      |
| 0–10 cm                                       | 12.9 $\pm$ 4.0       | 4.5 $\pm$ 3.4        | 11.8 $\pm$ 11.2      | 6.0 $\pm$ 3.0        |
| 0–40 cm                                       | 17.2 $\pm$ 5.4       | 6.0 $\pm$ 2.1        | 19.0 $\pm$ 18.1      | 8.3 $\pm$ 2.8        |

0–10 or 0–40 cm depths (Table 1). Cores from the brackish community at Newland Marsh generally had higher average organic carbon content than cores from the salt marsh community, although variability between cores was high, especially in the brackish community (Fig. 2B). There were no significant differences in organic carbon densities between brackish and salt marsh cores in Newland Marsh when calculated over the 0–10 or 0–40 cm depths (Table 1). Patterns of soil nitrogen through the depth profile mirrored organic carbon concentrations at both Magnolia and Newland Marshes (Fig. 2C and D), reflecting a strong relationship between organic carbon and nitrogen in the soils. There were no significant differences in total nitrogen density between vegetated and unvegetated cores in Magnolia Marsh or between brackish and salt marsh cores in Newland Marsh at either the 0–10 or 0–40 cm depth increments (Table 1).

Similar to organic carbon and total nitrogen, belowground biomass was generally higher in surface soils and decreased with depth (Fig. 3). Average total belowground biomass in both the 0–10 and 0–40 cm depth increments was nearly 3-times higher in the vegetated cores compared to the unvegetated cores in Magnolia Marsh; however, these differences were not statistically significant at either depth increment (Table 1). In Newland Marsh, average total belowground biomass in both the 0–10 and 0–40 cm depth increments was approximately twice as high in brackish cores compared to salt marsh cores, but these differences were not significant at either depth range (Table 1). Total organic carbon density in the 0–10 cm depth increased with increased belowground biomass in the same depth range ( $p=0.03$ ;  $r^2=0.48$ ; Fig. 4). There was no relationship between organic carbon density and belowground biomass in the 0–40 cm depth increment ( $p=0.63$ ; Fig. 4). Across all sites, organic carbon content increased with increasing concentrations of organic matter (measured as LOI;  $p<0.001$ ;  $r^2=0.96$ ; Fig. 5A.). Similarly, total nitrogen content was highest in samples with high organic matter content ( $p<0.001$ ;  $r^2=0.96$ ; Fig. 5B.).

### Discussion and Conclusions

Tidal influence had been restored at Magnolia Marsh for 3 years prior to sampling for this project and had yet to be restored at the nearby Newland Marsh. Despite different restoration histories, the upper 40 cm of soil in both sites stored between 0.013–0.015  $\text{g cm}^{-3}$

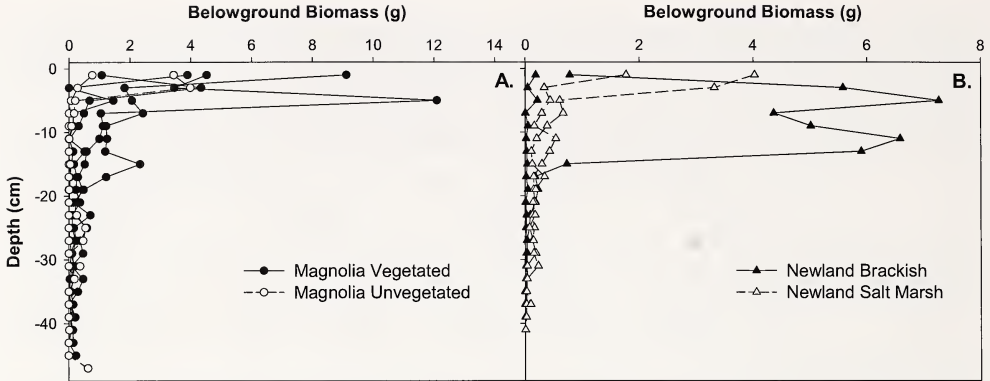


Fig. 3. Depth profiles of belowground biomass in Magnolia (A.) and Newland (B.) Marshes. Cores were collected as described in Figure 2.

of organic carbon (Table 1). These values are lower than the global average soil organic carbon density of  $0.039 \pm 0.003 \text{ g cm}^{-3}$  provided by Chmura et al. (2003). Soil organic carbon density measured in the current project was also lower than the values of  $0.034 \text{ g cm}^{-3}$  and  $0.023 \text{ g cm}^{-3}$  measured in the adjacent Brookhurst Marsh and Talbert Marsh which had been restored for 2 and 22 years, respectively (Keller et al. 2012). Taken together, these results verify our previous assertion that time since restoration does not appear to be the primary control of soil organic carbon content in this salt marsh landscape. This conclusion is in contrast to previous chronosequence studies which have documented increased soil carbon through time following restoration (e.g., Cornell et al. 2007; Craft et al. 2003).

However, Streever et al. (2000) suggested that inter-site differences in ecosystem properties may be greater than differences that emerge through time following restoration. We previously hypothesized that site-specific differences in pre-restoration vegetation may play a particularly important role in determining soil carbon density (or other soil conditions) at these sites (Keller et al. 2012). The current project provides limited support for this hypothesis. While there were trends towards higher soil carbon and nitrogen in the vegetated cores in Magnolia Marsh and the brackish cores in Newland Marsh (Fig. 2), these differences were not significant at either site (Table 1). It is worth noting that there was considerable spatial variability in soil properties even within a plant community type within the same marsh (especially in the brackish community in Newland Marsh). The reasons for this variability are unclear, but could include differences in marsh elevation, vegetation community and/or decomposition dynamics which are known to interact to influence carbon content and rates of soil accretion in marsh ecosystems (Kirwan and Megongial 2013). Future work should consider this variability when attempting to account for carbon storage within an entire marsh ecosystem.

Across both sites, 48% of the variability in soil organic carbon density in the upper 10 cm was explained by belowground biomass in the same depth interval (Fig. 4), suggesting that vegetation community can perhaps influence soil properties. Root and rhizome dynamics are rarely studied in wetland environments due to logistical constraints (e.g., Iversen et al. 2012), but these belowground processes may be important for understanding soil carbon and nitrogen dynamics. Decreasing belowground biomass with depth has been observed previously (Saunders et al. 2006) and may be driven by both biotic factors

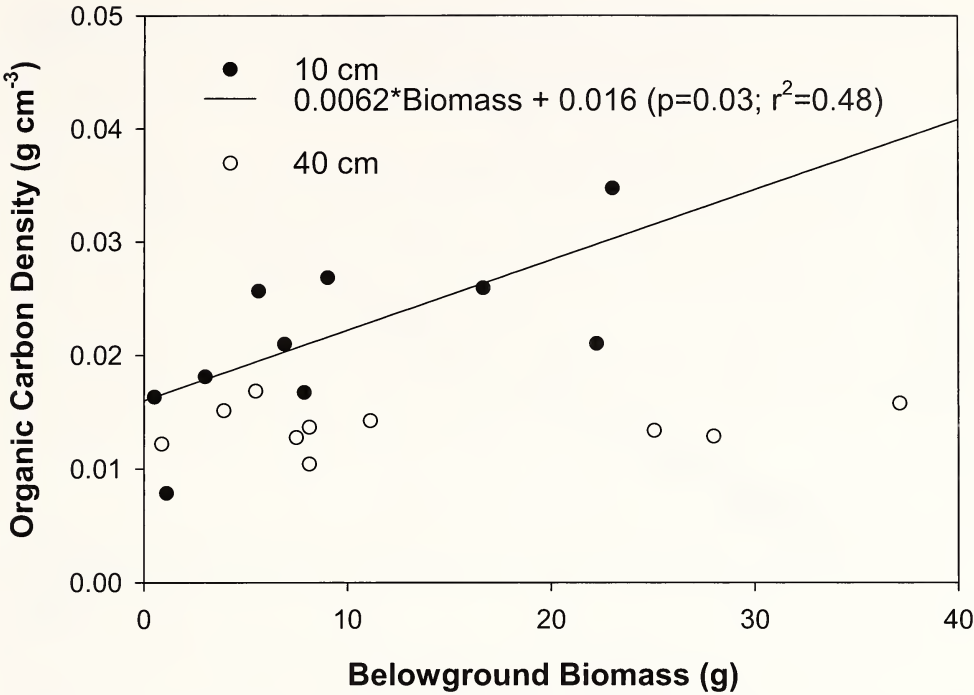


Fig. 4. Relationship between soil organic carbon density and belowground biomass in the upper 10 cm (closed symbols) and the upper 40 cm (open symbols) of salt marsh soil cores collected from both Magnolia and Newland Marshes.

(i.e., competition between species) and abiotic factors (i.e., flooding and oxygen availability or their interaction). Modeling approaches have explored the links between root productivity and soil carbon content (e.g., Mudd et al. 2009), and Langley et al. (2009) demonstrated that organic matter production in the form of fine roots in response to elevated atmospheric CO<sub>2</sub> was the primary driver of increased rates of accretion in a brackish marsh.

There was a strong relationship between soil organic carbon content and organic matter content (LOI) across all samples analyzed in the current project (Fig. 5). This relationship was similar to those reported by Craft et al. (1991) and Callaway et al. (2012) using salt and brackish marsh soils from North Carolina and San Francisco, California, respectively (Fig. 5), suggesting that this relationship is relatively robust across climate and vegetation types. The quadratic form of this relationship results from an increased fraction of organic carbon in organic matter in soils with higher organic matter contents. For example, organic matter from the 0–2 cm depth increment contained  $42 \pm 3$  (mean  $\pm 1$  SE) percent carbon compared to  $22 \pm 3$  percent carbon in organic matter from the 8–10 cm depth increment. These values are all below the 58% of organic matter predicted to be carbon based on the van Bemmelen factor (commonly used to convert organic matter to organic carbon) and are generally below the more recent estimate of 50% carbon suggested by Pribyl (2010). The deviations from these values are particularly pronounced in deeper (older) soils which might suggest that carbon is being lost from organic matter through time, perhaps through microbial respiration or through export of dissolved carbon.

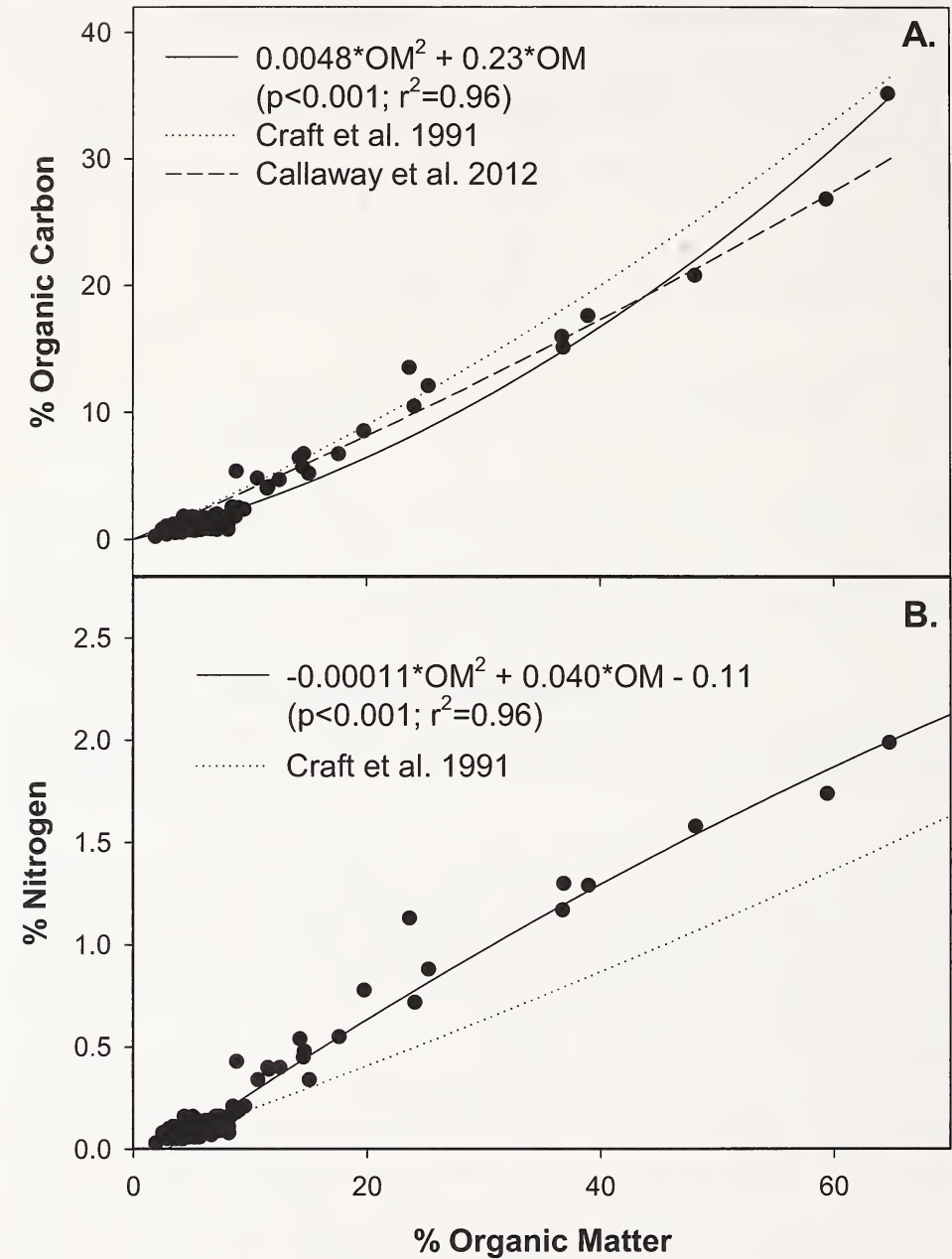


Fig. 5. Relationship between organic matter content (measured as loss on ignition, LOI) and organic carbon content (A.) and total nitrogen content (B.) in salt marsh soils collected from both Magnolia and Newland Marshes. Previously published relationships from Craft et al. (1991) and Callaway et al. (2012) are included for comparison.

Craft et al. (1991) also reported a relationship between total soil nitrogen content and organic matter content (LOI), suggesting that relatively simple measurements of LOI might provide indirect information on soil carbon and nitrogen. We also observed a strong relationship between soil nitrogen content and soil organic matter content (Fig. 5); however, our soils had a higher percent of soil nitrogen for a given organic

matter content (i.e., lower C:N) than those analyzed by Craft et al. (1991). Thus, while the relationship between organic carbon and organic matter appears to be robust across climates and vegetation types, the relationship between nitrogen and soil organic matter may be much more site-dependent and generalized relationships should be viewed with caution.

A lack of a consistent accumulation of soil organic carbon along a chronosequence of southern California salt marshes (from pre-restored to 22 years post-restoration) suggests that site-specific factors may be as important as time since restoration in controlling the “blue carbon” accumulation in these systems. Pre-restoration vegetation, as either the presence or absence of vegetation in Magnolia Marsh or as different vegetation communities in Newland Marsh, also did not play the key role in determining soil organic carbon (or total nitrogen) content in these marshes. However, a strong relationship between belowground biomass and soil organic carbon means that vegetation does likely play some part in determining soil properties.

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## Identical Response of Caged Rock Crabs (Genera *Metacarcinus* and *Cancer*) to Energized and Unenergized Undersea Power Cables in Southern California, USA

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Increasingly, energy generation facilities (i.e., wave and wind) are being sited in offshore marine waters. The electricity generated from these facilities is transmitted to shore through cables carrying alternating (AC) or direct (DC) current. If DC is used, it is converted to AC for the North American grid at onshore stations. While these currents produce both electric and magnetic fields, only the magnetic field, here called an electromagnetic field (EMF), is emitted from the cable. Some marine vertebrates and invertebrates can detect EMFs (summarized in Normandeau et al. 2011<sup>1</sup>). However, while it is clear that organisms can detect EMFs, less well understood is how these animals respond behaviorally to this stimulus, and concerns have been raised regarding how these organisms might interact with energized subsea cables<sup>1</sup>. Among fishes, a few field or quasi-field studies have produced what appear to be minor or equivocal responses. For instance, in a study of three species of elasmobranchs held in offshore mesocosms and subjected to EMF, there were some statistically significant differences in behavior; however these differences were inconsistent among individuals within a species<sup>2</sup>. In other studies, migrating European eels (*Anguilla anguilla*) in the Baltic Sea slowed, but did not halt, their swimming speed around an energized cable (Westerberg and Lagenfelt, 2008), and the movement of a number of fish species did not appear to be affected by an energized cable off Denmark<sup>3</sup>.

Along the Pacific Coast of the United States, fishers have also raised this issue<sup>4</sup>; one of the specific issues is how crabs (which form major fisheries along the Pacific Coast) might respond to energized power cables. There have been few studies on the behavioral changes that invertebrates might show in the presence of EMF although a small laboratory study implied that Dungeness crabs (*Metacarcinus magister*) were attracted to

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<sup>1</sup> Normandeau, Exponent, and T. Tricas, and A. Gill. 2011. Effects of EMFs from undersea power cables on elasmobranchs and other marine species. U.S. Dept. Int., Bur. Ocean Energy, Management, Regulation, and Enforcement, Pacific OCS Region, Camarillo, CA. OCS Study BOEMRE 2011-09.

<sup>2</sup> Gill, A.B., Y. Huang, I. Gloyne-Philips, J. Metcalfe, V. Quayle, J. Spencer, and V. Wearmouth. 2009. COWRIE 2.0 Electromagnetic Fields (EMF) Phase 2. EMF-sensitive fish response to EM emissions from sub-sea cables of the type used by the offshore renewable energy industry. COWRIE Ltd. COWRIE-EMF-1-06.

<sup>3</sup> DONG Energy and Vattenfall A/S. 2006. Review Report 2005 The Danish offshore wind farm demonstration project: Horns Rev and Nysted offshore wind farms environmental impact assessment and monitoring. The Danish Offshore Wind Farm Demonstration Projects.

<sup>4</sup> Pacific Fisheries Management Council (PFMC). 2010. Letter from PFMC to Federal Energy Regulatory Council, dated 19 June 2010. Titled COMMENT Reedsport OPT wave Park Project, FERC No. 12713. Accessed 11 December 2013. [http://www.pcouncil.org/wp-content/uploads/Cmt\\_Reedsport\\_OPT\\_FERC.pdf](http://www.pcouncil.org/wp-content/uploads/Cmt_Reedsport_OPT_FERC.pdf)

a zone of high EMF and that crabs in some zones with elevated EMF levels were somewhat more active than control animals<sup>5</sup>. Needed are studies that address how organisms respond to an *in situ* energized submarine power cable. The presence of energized and unenergized AC submarine cables in close proximity to one another off the coast of southern California allowed us to conduct such an experiment on crabs.

The experiments took place off Las Flores Canyon (34°28'N, 120°02'W), southern California, USA. Here several energized and unenergized submarine power cables, identical in construction, lie unburied on the seafloor and extend to offshore oil and gas platforms (Fig. 1). We selected two cables for this study; one was energized and the other unenergized. The two cables run parallel to each other, perpendicular to shore, and are approximately 7 m apart. Note that in an ongoing study we have determined that the EMF around the energized cable dissipates to background levels at a distance of about one meter.

We used stiff plastic perforated boxes (88 cm x 57 cm x 23 cm) that were secured to the sea floor with sand anchors at a bottom depth of 10 m. Each box was placed so that one end was in contact with one of the two cables. In all, twelve boxes were installed, six adjacent to the energized cable and six adjacent to the unenergized one. The boxes were installed at intervals of 2.5 meters along each cable, half on the east side and half on the west side and these alternated from one side to the other (Fig. 1). To reduce the chances of crabs visually sensing the cable, plastic panels were attached to the end of each box closest to the cable and identical panels were attached to the boxes on the end farthest from the cable. To further reduce the chances that the crabs could sense a difference between the cable end and the noncable end, we also removed the common brown macroalgae *Pterygophora californica* that occurs on the cables but does not live on the adjacent sea floor.

With the boxes in place along the energized and unenergized cables, divers stocked each with one adult crab of either *Metacarcinus anthonyi* or *Cancer productus*, for an experimental trial. Each crab, which was randomly selected from a stock of legal-sized crabs provided by a commercial crab fisherman, was dropped through a hinged hatch, which was centered in the middle of the cage. One hour after emplacement, divers recorded the position of the crab within the box by visually dividing it into two halves, the portion closest to the cable being designated “near-half” and that furthest from the cable “far-half” (Fig. 1). A second diver then opened the box to record EMF values (in microteslas -  $\mu$ T) with a handheld EMF detector (EMF 1390 from General Tools & Instruments). Readings were taken on the floor of each box at the edge closest to the cable and on the floor of that box furthest from the cable. The boxes were then leaving the crab in the box. Divers returned 24 hours later to observe where the crabs were positioned in the boxes and recorded EMF values. The crabs were then removed from the boxes and new, previously untested, crabs inserted for the next trial. Four sequential, 24-hour trials comprised an experiment. A total of four experiments were conducted in 2013 (10–14 June, 9–13 September, 30 September–4 October, and 7–11 October). Crabs were selected randomly for each box. Gender was recorded for each crab with exception of the first experiment.

The primary question we addressed in this study is whether crabs responded differently to the two types (energized and unenergized) of cables. The observations made 1 hour

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<sup>5</sup> Wilson, C.S. and D.L. Woodruff. 2011. A preliminary study on the effects of electromagnetic fields on the burial behavior and location of the Dungeness crab, *Cancer magister*. Pacific Northwest National Laboratory, Prepared for the U.S. Dept. Energy, Contract DE-AC05-76RL01830, PNNL-20729.

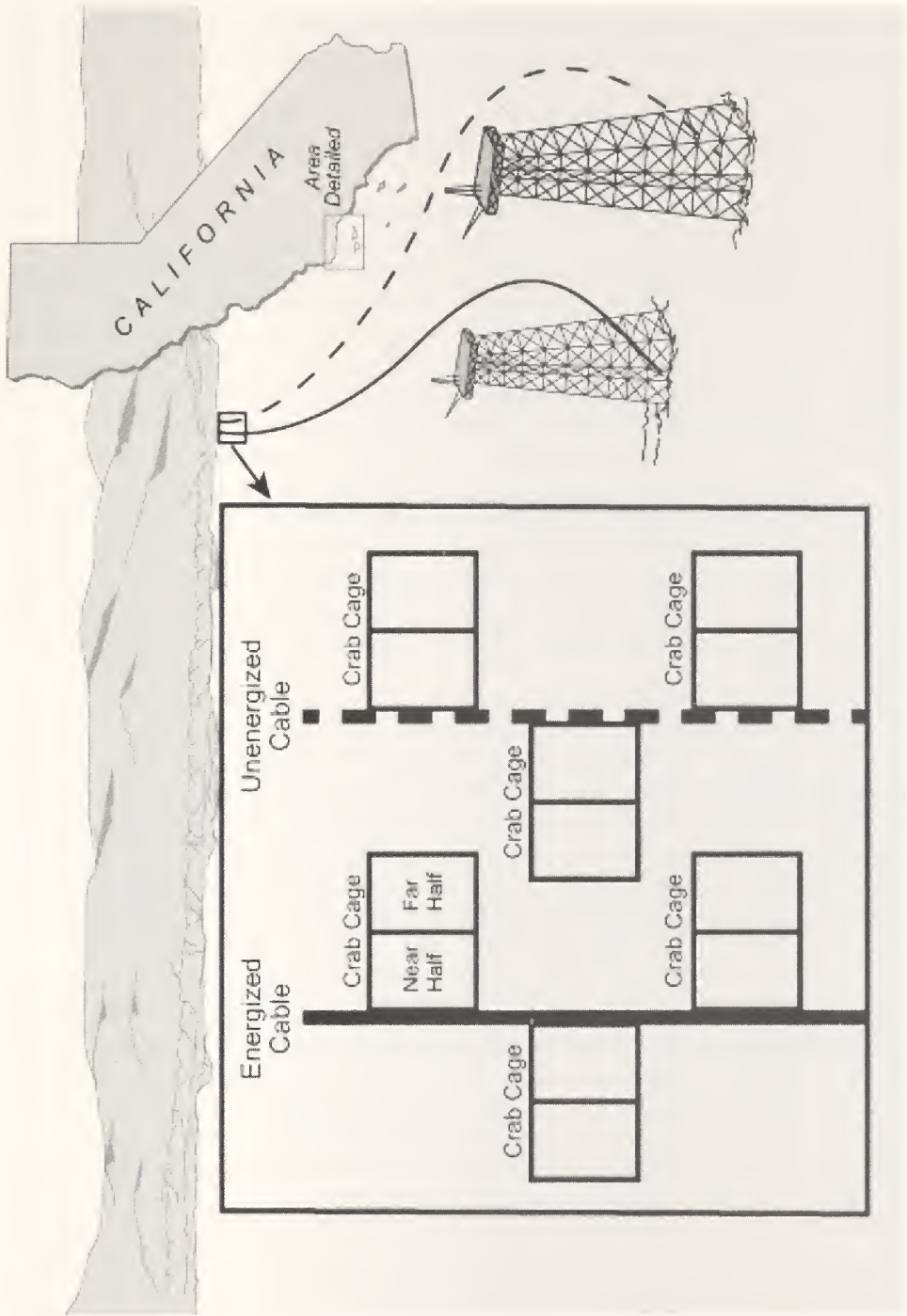


Fig. 1. The location of the energized and unenergized cables used in the experiments and the orientation of six of 12 boxes. The distance between the cables, about 7 m, is not drawn to scale.

and 24 hours after the crabs were set in the cages were evaluated separately. We used the generalized linear model (GLM) approach to determine if crabs along the energized cable were found nearer or farther from the cable compared to crabs along a non-energized cable. A crab's position, in the half of the box near or far from the cable, was the response variable. Given the binomial distribution of the response variable, a logistic regression model was used with a logit link function.

We used JMP software to fit each GLM to the data by Firth bias-adjusted maximum likelihood estimation of the parameter vectors<sup>6</sup>. The most complete GLM model analyzed included the effects of experiment (1–4), trial (1–4) nested within experiment, side of cable that the cage was set (west, east), and type of cable (energized and unenergized) as well as the intercept. A likelihood-ratio Chi-square test evaluated the hypothesis that all the model parameters were zero. We also examined a sequence of simpler GLM models to identify the best-fit model that might include as few as one predictor. Akaike's information criterion (AIC) was used to select between candidate models.

To determine if the genders responded differently to the energized and non-energized cables, we first added gender as a predictor in the complete GLM model using data from all but the first experiment when gender was not recorded. We used the same method above to determine the best-fit model. We also parsed the data by gender to determine if either male or female crabs, separately, responded differently to the two types of cables. Again, we used the same GLM approach described above to determine if cable type alone or with the other explanatory factors had a significant effect on a male or female crab's position in a box.

The EMF at the end of the boxes closest to the energized cable ranged from a mean of 46.2  $\mu\text{T}$  to 80.0  $\mu\text{T}$  during the experiments, and the readings on the far end of the boxes never exceeded 0.9  $\mu\text{T}$  (Table 1). Along the unenergized cable, EMF did not exceed 0.2  $\mu\text{T}$  in the near half or far half of the boxes during the experiments. A total of 192 crabs were used in this study; 24 crabs in each of four experiments on each cable (Table 2). The positions of all 192 crabs were observed 1 hour after emplacement. A total of eight crabs were recorded as lost 24 hours after emplacement during the four experiments; three crabs in boxes along the unenergized cable and five crabs along the energized cable. Escapement was not possible and loss of crabs was likely due to predation by octopuses.

The crabs responded no differently in the boxes along the unenergized and energized cables. Both 1-hour and 24-hours after the crabs were set in the boxes, there were no apparent differences in the proportion of crabs near the two types of cable regardless of the side of cable where the boxes were set (Fig. 2). For a given observation period, experiment, trial nested within experiment, side of cable that the cage was set, and type of cable had no significant effect on the position of crabs in the boxes as evident from the GLM that was not significantly different from the intercept model (1 hour:  $n=192$ ,  $-\log \text{likelihood} = 5.676$ ,  $X^2=11.351$ ,  $\text{DF}=17$ ,  $p=0.838$ ,  $\text{AIC}=295.901$ . 24 hours:  $n=184$ ,  $-\log \text{likelihood} = 7.946$ ,  $X^2=15.892$ ,  $\text{DF}=17$ ,  $p=0.532$ ,  $\text{AIC}=281.037$ ). None of the GLMs that incorporated fewer explanatory factors could predict with statistical significance the variability in crab responses in the boxes next to the cables one hour or 24 hours after deployment.

The proportion of crabs near the two types of cables 24 hours after deployment was highly variable across experiments regardless of side of the cable the box was set (Fig. 2).

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<sup>6</sup> Schwarz, C.J. 2013. Sampling, regression, experimental design and analysis for environmental scientists, biologists, and resource managers. <http://people.stat.sfu.ca/cschwarz/Stat-650/Notes/PDFbigbook-JMP/>.

Table 1. Level of electromagnetic field (microteslas -  $\mu$ T) in those parts of boxes *closest* to unenergized and energized cables as read one hour and 24 hours after crabs were inserted. EMF readings at the farthest end of the boxes were  $<0.1\mu$ T at the unenergized cable and  $<0.9\mu$ T at the energized cable.

The lower n in experiments 1 and 4 were due to the flooding of the housing containing the EMF meter after the first day of observations, which led to failure of the devices. However, note that the energized cable used in this experiments has been in continuous use for many years and did not fail during the course of these studies.

| Experiment | Cable Type  | 1 hr      |      |    | 24 hr     |      |    |
|------------|-------------|-----------|------|----|-----------|------|----|
|            |             | $\bar{x}$ | sd   | n  | $\bar{x}$ | sd   | n  |
| 1          | Unenergized | 0.0       | 0.0  | 6  | –         | –    | 0  |
|            | Energized   | 46.2      | 11.4 | 6  | –         | –    | –  |
| 2          | Unenergized | 0.0       | 0.0  | 24 | 0.1       | 0.0  | 24 |
|            | Energized   | 57.0      | 7.4  | 24 | 55.5      | 8.7  | 24 |
| 3          | Unenergized | 0.0       | 0.0  | 24 | 0.1       | 0.0  | 24 |
|            | Energized   | 54.2      | 9.3  | 24 | 56.1      | 0.0  | 24 |
| 4          | Unenergized | 0.1       | 0.0  | 6  | 0.1       | 0.1  | 6  |
|            | Energized   | 80.0      | 19.7 | 6  | 51.0      | 10.1 | 6  |

Combining the observations from the four experiments, the proportion of crabs found close to the two types of cable changed little between the observations made one hour and 24 hours after the crabs were set in the boxes (Fig. 3). One hour after emplacement, 53% (51 of 96) of the crabs set along the unenergized cable and 55% (53 of 96) of the crabs along the energized cable were observed in the near-half of the boxes (Fig. 3). The log-likelihood test of the GLM showed no cable-type effect on crab response ( $n=192$ ,  $-\log$  likelihood=0.042,  $\chi^2=0.084$ ,  $DF=1$ ,  $p=0.772$ ,  $AIC=270.876$ ). The AIC for this single-factor model indicates that it is no worse fit of the 1-hour data than the GLM of all explanatory factors. In comparison, 24 hours after emplacement 56% (52 of 93) of the crabs set along the unenergized cable and 51% (46 of 91 of the crabs set along the energized cable were in the near-half of the boxes (Fig. 3). Although a slightly greater proportion of crabs were nearer the unenergized cable than the energized cable,

Table 2. Number and gender (F = female, M = males, Unk = unknown) of crabs used in four experiments. Gender of crabs in experiment 1 was not determined. Loss of crabs between one hour and 24 hours was likely due to predation by octopuses.

|              | Unenergized |   |     |       | Energized |   |     |       | Grand Total |
|--------------|-------------|---|-----|-------|-----------|---|-----|-------|-------------|
|              | F           | M | Unk | Total | F         | M | Unk | Total |             |
| Experiment 1 |             |   |     |       |           |   |     |       |             |
| 1 hr         |             |   | 24  | 24    |           |   | 24  | 24    | 48          |
| 24 hrs       |             |   | 23  | 23    |           |   | 24  | 24    | 47          |
| Experiment 2 |             |   |     |       |           |   |     |       |             |
| 1 hr         | 17          | 7 |     | 24    | 17        | 7 |     | 24    | 48          |
| 24 hrs       | 17          | 7 |     | 24    | 17        | 7 |     | 24    | 48          |
| Experiment 3 |             |   |     |       |           |   |     |       |             |
| 1 hr         | 17          | 7 |     | 24    | 22        | 2 |     | 24    | 48          |
| 24 hrs       | 15          | 7 |     | 22    | 19        | 2 |     | 21    | 43          |
| Experiment 4 |             |   |     |       |           |   |     |       |             |
| 1 hr         | 18          | 6 |     | 24    | 17        | 7 |     | 24    | 48          |
| 24 hrs       | 18          | 6 |     | 24    | 16        | 6 |     | 22    | 46          |

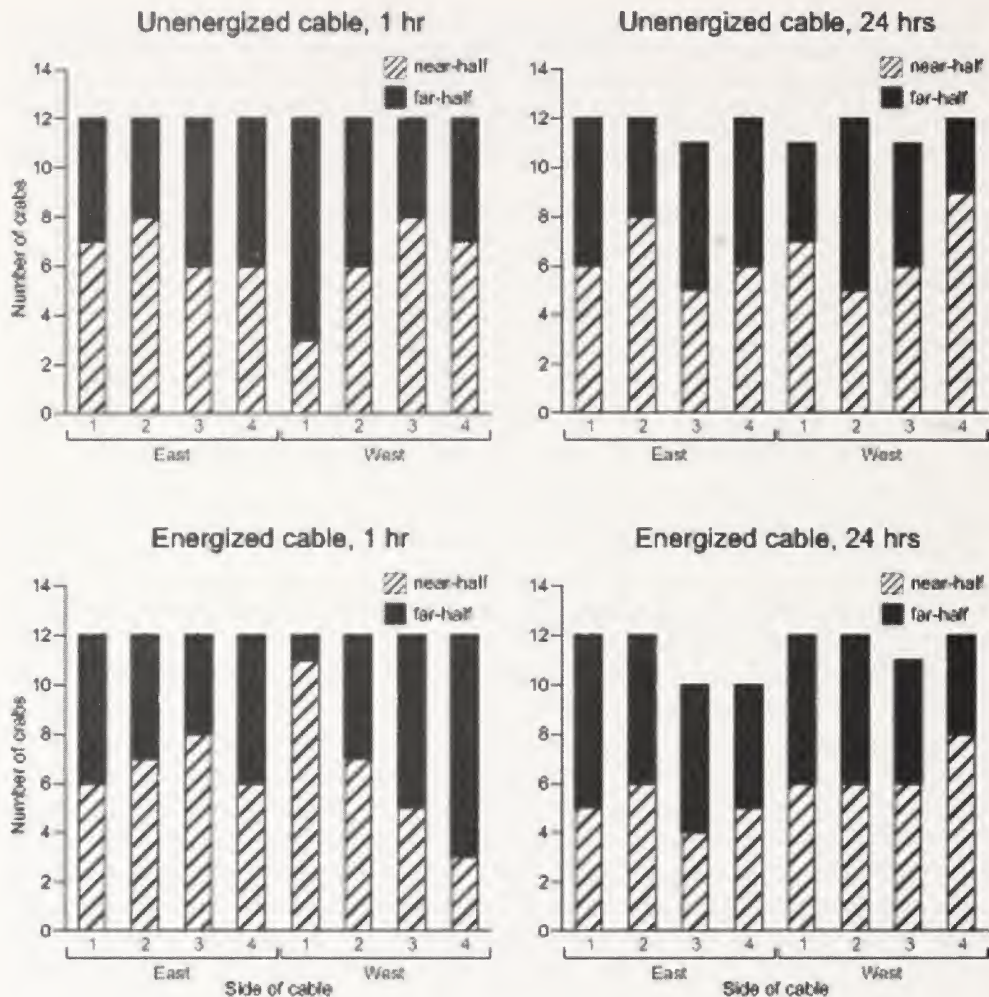


Fig. 2. The number of crabs positioned in the near-half and far-half of boxes on the west side and east side of the energized and unenergized cables by experiment, one hour and 24 hours after deployment.

cable type in the single factor model had no effect on crab response ( $n=184$ ,  $-\log$  likelihood = 0.266,  $\chi^2=0.5318$ ,  $DF=1$ ,  $p=0.466$ ,  $AIC=259.897$ ). As was the case for the 1-hour observations, the proportion of crabs found close to the two types of cable did not differ 24 hours after the crabs were set in the boxes.

Some of the crabs were found in the opposite half of the box when reexamined 24 hours later. Along the energized cable, 23.1% (21 individuals) of 91 crabs moved to the half of the box that was closer to the cable from the half farther, and 27.5% (25 individuals) moved to the half of the box farther from the half nearer. Along the non-energized cable, 21.5% (20 of 93 individuals) moved to half of the box that was closer to the cable, and 18.3% (17) moved to the farther half of the box. Movement of crabs within the boxes between the one-hour and 24-hour observations is unknown.

The addition of gender to the complete GLM faired no better using data from experiments 2-4 when gender was recorded (1 hour:  $n=144$ ,  $-\log$  likelihood = 6.632,  $\chi^2=13.265$ ,  $DF=14$ ,  $p=0.506$ ,  $AIC=221.950$ . 24 hours:  $n=137$ ,  $-\log$  likelihood = 7.136,

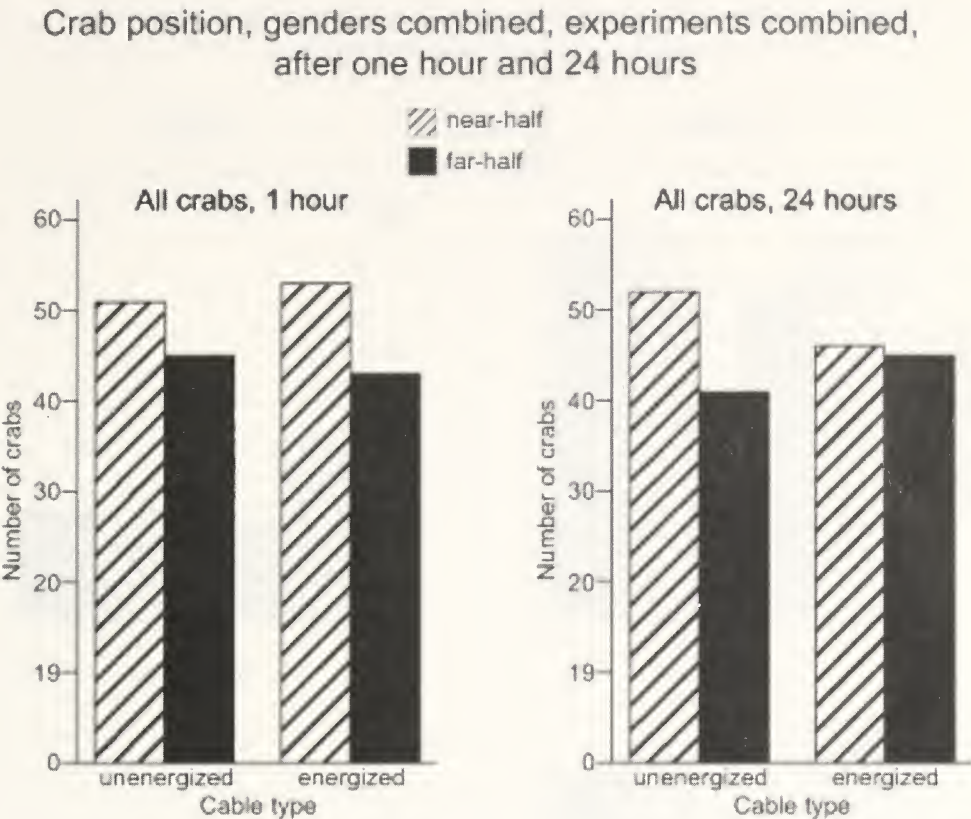


Fig. 3. The number of crabs positioned in the near-half and far-half of boxes adjacent to energized and unenergized cables after one and 24 hours.

$\chi^2=14.272$ ,  $DF=14$ ,  $p=0.430$ ,  $AIC=211.300$ ). None of the GLMs that incorporated fewer explanatory factors could predict with statistical significance the variability in crab responses in the boxes next to the cables one hour or 24 hours after deployment.

Specifically, cable type had no effect on a crab's position in the boxes regardless of gender (Fig. 4). One hour after emplacement, 54% of the females next to the unenergized cable (26 of 52 crabs) and 50% of the females next to the energized cable (28 of 56) were found in the near half of boxes ( $n=108$ ,  $-\log \text{likelihood} = 0.080$ ,  $\chi^2=0.160$ ,  $DF=1$ ,  $p=0.689$ ,  $AIC=155.643$ ). Twenty-four hours later, a slightly higher proportion of crabs were found next to both types of cables, 58% of the females (29 of 50 crabs) next to the unenergized cable were found in the near-half of boxes, whereas 52% of the females set along the energized cable (27 of 52 crabs) were in the near-half. Again, the females responded no differently to the two cable types ( $n=102$ ,  $-\log \text{likelihood} = 0.190$ ,  $\chi^2=0.380$ ,  $DF=1$ ,  $p=0.538$ ,  $AIC=146.285$ ). Males also responded no differently to the two cable types. One hour after emplacement, 65% of the males next to the unenergized cable (13 of 20 crabs) and 50% of the males next to the energized cable (8 of 16) were found in the near half of the boxes (Fig. 4). Although it appears that a greater proportion of males were found nearer the unenergized cable than energized cable, cable type in the single factor GLM had no statistically significant effect on male crab response ( $n=36$ ,  $-\log \text{likelihood} = 0.410$ ,  $\chi^2=0.820$ ,  $DF=1$ ,  $p=0.365$ ,  $AIC=54.8330$ ). Twenty-four hours

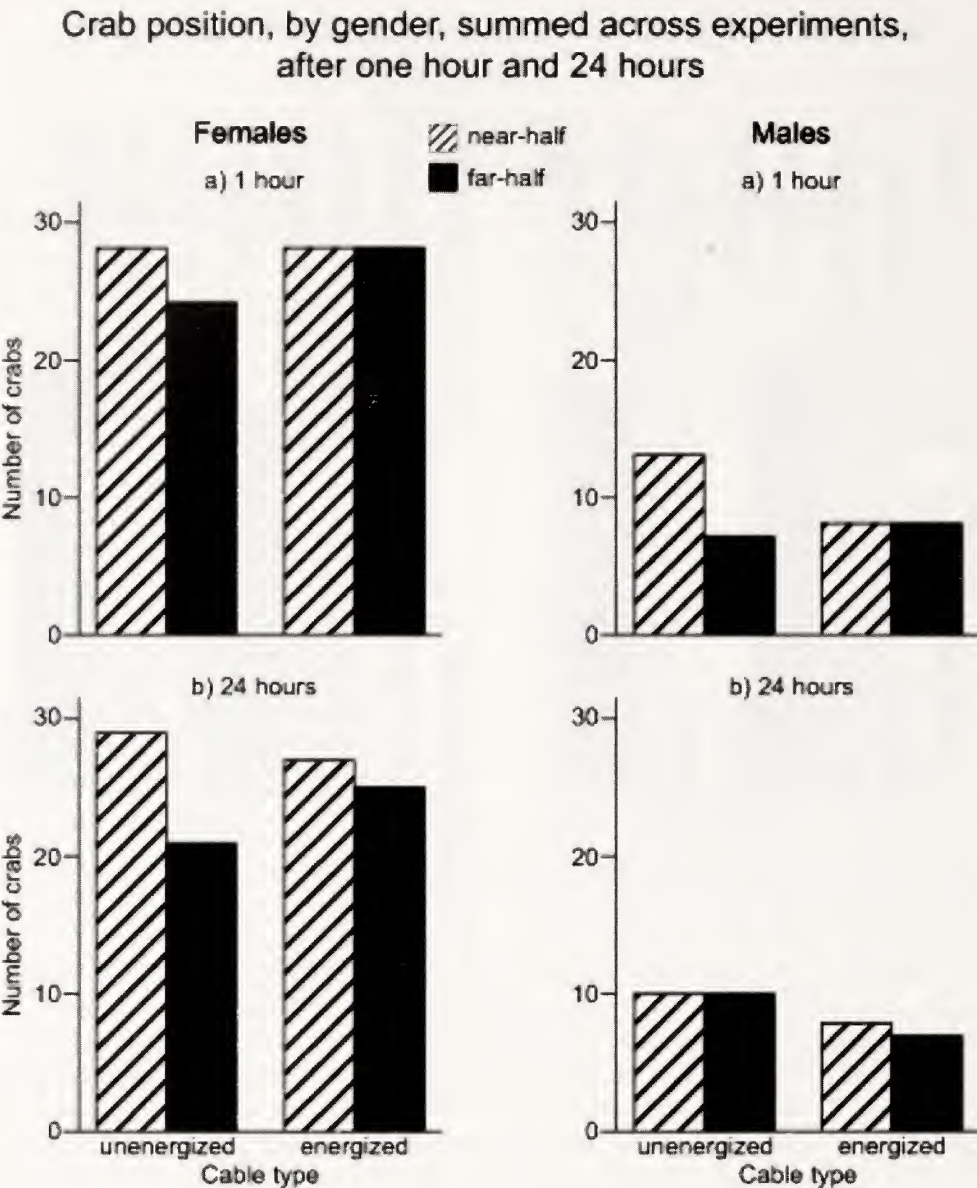


Fig. 4. The number of female and male crabs positioned in the near-half and far-half of boxes adjacent to energized and unenergized cables, one hour and 24 hours after deployment.

later, 50% of the males next to the unenergized cable (10 of 20 crabs) and 53% of the males next to the energized cable (8 of 15) were found in the near half of the boxes ( $n=35$ ,  $-\log \text{likelihood} = 0.019$ ,  $\chi^2 = 0.038$ ,  $DF=1$ ,  $p=0.846$ ,  $AIC=55.228$ ).

Pacific Coast crab fishers have voiced several concerns regarding crabs and their potential responses to the EMF generated by submarine power cables. These concerns generally relate to whether crabs are either attracted to, or repulsed by, EMF. If either of these occurs, crab migrations might be compromised and, more specifically, crabs might not walk over a cable to reach a baited trap. While this experiment does not address all of

these concerns, it does imply that these two crab species may not respond either positively or negatively to the levels of EMF generated by this specific energized cable.

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## Recent Decline of Lowland Populations of the Western Gray Squirrel in the Los Angeles Area of Southern California

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**Abstract.**—We provide an overview of the distribution of lowland and otherwise isolated populations of the western gray squirrel (*Sciurus griseus*) in the Los Angeles area of southern California, an area that has experienced a recent and ongoing invasion by the non-native eastern fox squirrel (*Sciurus niger*), an urban-adapted species introduced a century ago. Away from its strongholds in the western Santa Monica Mountains, San Gabriel Mountains, and Santa Ana Mountains, the western gray squirrel is resident locally in both the Santa Susana and the Verdugo Mountains, in Griffith Park, in low hills at the eastern periphery of the San Gabriel Valley and in Claremont, and along the Santa Ana River canyon near Yorba Linda. It also persists east of the Los Angeles area in residential areas of Redlands and Yucaipa, which as of 2014 are still outside the range of the eastern fox squirrel. Here we document several gray squirrel extirpation events within its lowland range, and discuss factors influencing its persistence and its extirpation.

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The western gray squirrel (*Sciurus griseus*) is a large tree squirrel native to forests of the western United States and extreme northwestern Mexico, with the subspecies *S. g. anthonyi* common and widespread in oak- and pine-dominated areas of the hills and mountains of southern California (Wilson and Reeder 2005). In the Los Angeles area, a region we define as extending from eastern Ventura County east through Claremont and south through the coastal plain into Orange County to the base of the San Joaquin Hills, it also occurs in human-modified habitats, including large city parks and golf courses, where scattered trees, particularly conifers, provide year-round food and shelter. It is one of two tree squirrels in the Los Angeles area, the other being the eastern fox squirrel (*Sciurus niger*), a non-native introduced in the early 1900s, and now abundant throughout much of the Los Angeles area of southern California (Jameson and Peeters 1988, King et al. 2010).

As discussed by Linders and Stinson (2007) western gray squirrels are closely tied to oak and evergreen woodland, and serve two main roles in maintaining native woodlands: they harvest and bury acorns throughout the woodland, and disperse the seeds and fruit of various oak woodland component tree and shrub species, such as California bay (*Umbellularia californica*). They also forage heavily on truffle-like mycorrhizal fungi found in leaf litter and loose soil, which aid oaks in fixing nitrogen and retaining water through dry months. During foraging, western gray squirrels deposit the spores of these fungi through their droppings, thus spreading them throughout the oak woodland and promoting the health of its trees. Because of this close association with oaks, the presence

of western gray squirrels may serve as an indicator of oak woodland health. By contrast, the eastern fox squirrel is highly generalist in its food sources, requires a much smaller home range (becoming super-abundant in urban settings), and occurs in a much wider array of habitats than *S. griseus* away from the major mountain ranges in the region (Gatza 2011, Ortiz 2014).

The history and origin of western gray squirrel populations on the floor of the Los Angeles Basin are poorly understood. Today, most lowland populations of *S. griseus* are strongly associated with planted pines and other conifers, which may now be crucial habitat elements for the species. It was presumably naturally present at lower elevations when oak woodland (mostly *Quercus agrifolia*) once covered large areas of now-urbanized places like the San Gabriel Valley, a pattern shared by numerous lower montane plant and wildlife species that are able to persist locally at lower elevations in suitable areas of canyons and woodlands (Cooper 2011). Later, as the region developed, populations of *S. griseus* may have retreated to large urban parks and more wooded residential areas, where it persisted through most of the 1900s, including those at the base of the San Gabriel Mountains foothills from Pasadena east into Claremont (an area referred to as the “mesa” by early naturalists, e.g., Grinnell 1898). It is also possible that they colonized these areas later by moving down from the surrounding foothills, or that both patterns occurred, with isolated lowland populations “winking” out periodically, replenished by animals from surrounding highlands. Whatever the history, in the years between the late 1990s and the mid-2000s, *S. griseus* became scarce or altogether absent within many of these same neighborhoods. Clear instances of its extirpation and subsequent replacement – directly or indirectly – by the non-native eastern fox squirrel are now well documented (e.g., Muchlinski et al. 2009, Guthrie 2009, King et al. 2010).

*Sciurus niger* became established in the neighborhoods surrounding the eastern Santa Monica Mountains in the western Los Angeles Basin during the decades following its introduction in 1904, it only arrived in the San Gabriel Valley around 1990, the east San Gabriel Valley around 1998, and the Claremont area and Orange County in the early 2000s (Guthrie 2009, King et al. 2010). In recent years, *S. niger* has also colonized much of urbanized Santa Barbara County (P. Collins, pers. comm.) and portions of San Diego County, the latter also following an early introduction (King et al. 2010). Now virtually ubiquitous throughout the Los Angeles area from the San Fernando Valley east to San Bernardino County and south through Orange County, *S. niger* appears to still be absent at several urban-edge locations at the margins of the Los Angeles area, including parks and neighborhoods in Redlands and Yucaipa, San Bernardino Co. (Ortiz 2014); canyons in the lower San Gabriel Mountain foothills from the Sunland-Tujunga area east through Claremont (Gatza 2011), and along the Santa Ana River at Gypsum Canyon, near Yorba Linda, Orange Co. (AEM, unpubl. data).

Only a handful of local naturalists have noticed this turnover, and few published data exist on the range of *S. griseus* in the Los Angeles area prior to the arrival of *S. niger*. Today, only a few populations of *S. griseus* remain away from the larger mountains [typically below around 457 m (1500') a.s.l.], with only a handful, at the far eastern periphery being free of *S. niger*. To ensure the ecological integrity of these remaining populations of *S. griseus* – and of their habitat patches – particularly in areas where *S. niger* has not yet invaded (or at least where it is not completely dominant), it is important that remaining populations of *S. griseus* be identified and recognized by conservation agencies and organizations. Since the late 1990s, we (DSC and AEM) have been making notes on the occurrence of *S. griseus* in the Los Angeles area, as described

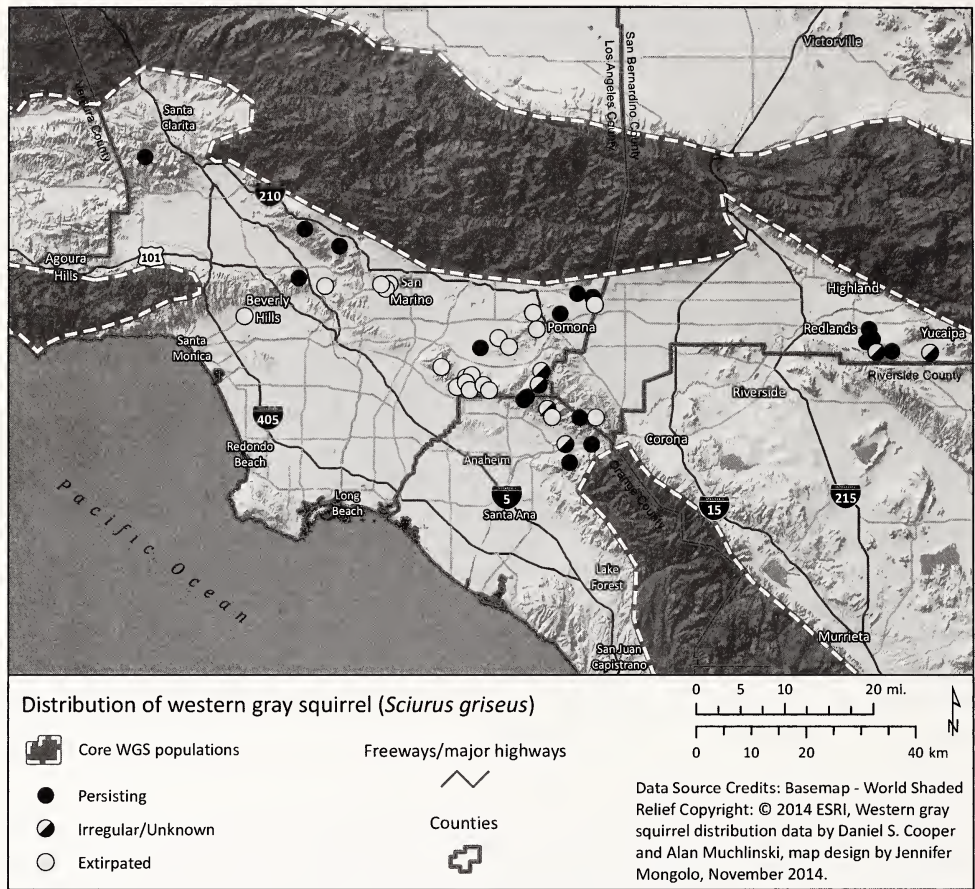


Fig. 1. Map showing current (2012–2014) range of western gray squirrel in the Los Angeles area.

below. This paper synthesizes findings from each of these efforts and provides detail on a dramatic and ongoing ecological replacement of a native species by a non-native one.

Materials and Methods

Little published information exists on the current or historical distribution of the western gray squirrel, so we relied on a variety of sources, including online museum databases for specimen records ([www.vertnet.org](http://www.vertnet.org), last search conducted 21 October 2014), and field notes and recollections of a network of environmental professionals and colleagues in the Los Angeles area. DSC conducted surveys of birds and vegetation in the Puente and Chino Hills on the east side of the Los Angeles Basin for two years in the late 1990s (1997–1998; see Cooper 2000), and kept field notes of all sightings of *S. griseus* from this area. AEM collected data on observations of *S. griseus* through an online survey form (<http://instructional1.calstatela.edu/amuchli/squirrelform2.htm>), which has received over 9000 visits since January 23, 2007, through field studies by four graduate students (Lewis 2009, Gatza 2011, Erkabaeva 2013, Ortiz 2014), and through his own observations within and east of the San Gabriel Valley.

DSC initiated a volunteer-based tree squirrel survey of Griffith Park in summer 2010; with ten observers each searching up to five of 40 similarly sized survey blocks in and

around the park. The following year, DSC and volunteers spent 25 days in the park between 8 August and 21 November 2011, gathering observations on foraging, breeding, aggression displays and other behavior, and from 5 August 2011 to 11 July 2012, DSC conducted a region-wide search for any remaining *S. griseus* populations in the lowland Los Angeles area away from known occupied habitat. During this period, DSC posted short articles and requests for information on local listserves (e.g., Pasadena Audubon Society; various neighborhood “Patch” websites). Also, DSC and colleagues made site visits (30 min – 2.5 hrs in duration) on 21 dates to 32 different locations in the eastern Santa Monica Mountains, the west San Gabriel Valley, and in the Verdugo Mountains and San Rafael Hills north of Glendale following up on reports and checking all accessible lowland areas with appropriate habitat. To supplement these surveys, DSC and colleagues installed motion-activated cameras during the same time period at Descanso Gardens in the San Rafael Hills west of Pasadena (two near the upper portion of the property bordering open space) and in the Verdugo Mountains (nine within canyons in three areas: La Tuna Canyon, Cedarbend Canyon, and Whiting Woods; *ibid*).

### Results

Within its core range in mountains at the periphery of the Los Angeles area, *Sciurus griseus* is a conspicuous resident in canyons and oak groves, and appears to have little contact with *S. niger* except at the immediate urban-wildland interface zone (Gatza 2011). Below around 457 m (1500') a.s.l., numerous subpopulations of *S. griseus* persisted into the 1990s in areas between these major mountain ranges, and in some cases, well onto the floor of urbanized areas, as summarized below, and in Figure 1 and Table 1.

#### *Eastern Santa Monica Mountains/Griffith Park*

Griffith Park, at the far eastern end of the Santa Monica Mountains, appears to support the only large remaining population of the species in this range east of Sepulveda Pass/Interstate 405, with approximately 25–50 individuals largely confined to two main drainages (Western Canyon and Vermont Canyon). During intensive searches of potential habitat in 2011 and 2012, no observations of *S. griseus* were made between Sepulveda Pass and Cahuenga Pass (U.S. 101), an area that includes significant open space at Franklin Canyon Park and elsewhere. However, we remain hopeful that *S. griseus* may persist here, as we were unable to obtain access into the large Stone Canyon Reservoir open space (Los Angeles Dept. of Water and Power) just east of Sepulveda Pass near Bel Air, which supports apparently suitable habitat.

#### *Santa Susana Mountains/Simi Hills*

Located on the northwestern edge of the San Fernando Valley, these generally arid ranges are dominated by low-growing chaparral and coastal sage scrub, with a small number of permanent streams and oak woodlands, best developed in the former range. Ecologically, the Simi Hills are more similar to the Santa Monica Mountains immediately to the south than the San Gabriel and Sierra Madre ranges to the north, while the Santa Susana Mountains reach higher elevations and feature more montane elements such as bigcone douglas-fir (*Pseudotsuga menziesii*) and extensive savannah dominated by annual grassland and valley oaks (*Quercus lobata*). Based on field notes of local naturalists, *S. griseus* is absent from the Simi Hills, but persists in the Santa Susana Mountains at Browns Canyon and Devils Canyon (S. Bernal, 2012, in litt.), and possibly at O'Melveny Park (sight record on 20 April 2014, CSULA web survey). Its historical status in either

Table 1. Distribution of “lowland” populations of western gray squirrel in Los Angeles area (i.e., away from major mountain ranges/foothills), 2014. Status: P = Persisting population; E = Extirpated; EFS = Eastern fox squirrel.

| Region                                       | Subarea                           | Location,<br>as applicable     | Elevation | Status | EFS? | Date of last<br>record (Source)           |
|----------------------------------------------|-----------------------------------|--------------------------------|-----------|--------|------|-------------------------------------------|
| East Santa Monica Mountains                  |                                   |                                |           |        |      |                                           |
|                                              | Beverly Hills                     |                                | <1200'    | E      | Yes  | 1975 (specimen, LACM 60617)               |
|                                              | Griffith Park                     |                                | <1200'    | P      | Yes  | 2014                                      |
| Santa Susana Mountains                       |                                   |                                |           |        |      |                                           |
|                                              |                                   |                                | 1400'     | P      | Yes  | 2014 <sup>1,2</sup>                       |
| West San Gabriel Valley                      |                                   |                                |           |        |      |                                           |
|                                              | Verdugo Mountains                 |                                | 1800'     | P      | Yes  | 2012 (DSC, unpubl. data)                  |
|                                              | San Rafael Hills                  |                                | 1300'     | P      | Yes  | 2014 (Erkabaeva 2013)                     |
|                                              | San Marino                        |                                |           |        |      |                                           |
|                                              |                                   | Huntington Library             | 600'      | E      | Yes  | 2010 (CSULA database)                     |
|                                              |                                   | Lacy Park                      | 600'      | E      | Yes  | 1976 (specimen, LACM 90234)               |
|                                              |                                   | Mission Canyon <sup>3</sup>    | 600'      | E      | Yes  | 2012 (CSULA database) <sup>4</sup>        |
| Northeast Los Angeles (Forest Lawn Glendale) |                                   |                                |           |        |      |                                           |
|                                              |                                   |                                | 600'      | E      | Yes  | 1997 (DSC, unpubl. data)                  |
| East San Gabriel Valley                      |                                   |                                |           |        |      |                                           |
|                                              | San Jose Hills                    |                                |           |        |      |                                           |
|                                              |                                   | Industry Hills                 | 600'      | P      | Yes  | 2014 (AEM, unpubl. data)                  |
|                                              |                                   | Bonelli Park area <sup>5</sup> | 1000'     | P      | Yes  | 2014 (AEM, unpubl. data)                  |
|                                              |                                   | Walnut Creek Park              | 800'      | E      | Yes  | 2012 (DSC, AEM, unpubl. data)             |
|                                              |                                   | Galster Park                   | 600'      | E      | Yes  | 1998 (DSC, unpubl. data)                  |
|                                              |                                   | Cal Poly Pomona                | 800'      | E      | Yes  | 2009 (AEM 2009)                           |
|                                              |                                   | Via Verde Country Club         | 800'      | E      | Yes  | ~2000 (AEM, unpubl. data)                 |
|                                              | Western Puente Hills <sup>6</sup> |                                |           |        |      |                                           |
|                                              |                                   | Whittier/Hacienda Heights      | 800'      | E      | Yes  | 1998 (DSC, unpubl. data)                  |
|                                              |                                   | Powder Canyon                  | 800'      | E      | Yes  | 2005 (R. Erickson, unpubl. data)          |
|                                              | Eastern Puente Hills/Chino Hills  |                                |           |        |      |                                           |
|                                              |                                   | Tonner Canyon                  | 600–800'  | P      | Yes  | 2014 (R. Hamilton, L. Schmahl, via email) |
|                                              |                                   | Chino Hills State Park         | 1200'     | P      | Yes  | 2014 (A. Ing, pers. comm.)                |
| Pomona Valley/Claremont                      |                                   |                                |           |        |      |                                           |
|                                              |                                   | RSABG <sup>7</sup>             | 1200'     | P      | Yes  | 2014 (AEM, unpubl. data)                  |
|                                              |                                   | Pomona College                 | 1200'     | E      | Yes  | 2012 (AEM, unpubl. data)                  |
|                                              |                                   | Arlington Dr.                  | 1200'     | P      | Yes  | 2012 (CSULA database)                     |
| Redlands/Yucaipa                             |                                   |                                |           |        |      |                                           |
|                                              | North of I-10                     |                                |           |        |      |                                           |
|                                              |                                   | Univ. of Redlands/Sylvan Park  | 1500'     | P      | No   | 2014 (Ortiz 2014)                         |
|                                              |                                   | 3 <sup>rd</sup> St., Yucaipa   | 2600'     | ?      | No   | 2011 (CSULA database)                     |
|                                              | South of I-10                     |                                |           |        |      |                                           |
|                                              |                                   | Ford Park                      | 1600'     | P      | No   | 2014 (Ortiz 2014)                         |
|                                              |                                   | Prospect Park                  | 1600'     | P      | No   | 2014 (Ortiz 2014)                         |
|                                              |                                   | Rossmont Dr.                   | 2000'     | ?      | No   | 2009 (CSULA database)                     |
|                                              |                                   | Hilltop Dr.                    | 2200'     | P      | No   | 2012 (CSULA database)                     |

Table 1. Continued.

| Region        | Subarea                | Location,<br>as applicable  | Elevation | Status | EFS? | Date of last<br>record (Source) |
|---------------|------------------------|-----------------------------|-----------|--------|------|---------------------------------|
| Orange County |                        |                             |           |        |      |                                 |
|               | Anaheim Hills          |                             |           |        |      |                                 |
|               |                        | Oak Canyon Nature<br>Center | 800'      | P      | Yes  | 2013 (CSULA database)           |
|               | Santa Ana River Canyon |                             |           |        |      |                                 |
|               |                        | Yorba Reg. Park             | 400'      | P      | Yes  | 2014 (B. Leatherman, via email) |
|               |                        | Canyon RV Park              | 400'      | P      | No   | 2014 (AEM, unpubl. data)        |

<sup>1</sup> Includes sight record from O'Melveny Canyon Park in 2014 (CSULA web survey).  
<sup>2</sup> S. Bernal, unpubl. data.  
<sup>3</sup> Includes oak woodland patches along Kewen, Canon and Encino Dr. at San Marino/Pasadena border.  
<sup>4</sup> This population was seen continuously through 2010; the 2012 report was likely a dispersing individual from elsewhere.  
<sup>5</sup> Includes Mountain Meadows Golf Course.  
<sup>6</sup> We use State Route 57 as the east/west dividing line.  
<sup>7</sup> Rancho Santa Ana Botanic Garden, Claremont.

range is not known (a single specimen exists from “Oat Mountain” from 1969, LACM 47332), nor is the size of the extant population in the Santa Susana Mountains. A recent (2014) observation of a roadkill *S. griseus* on U.S. 101 at Las Virgenes Canyon Rd. (C. DeMarco, via email) suggests that colonization north from the Santa Monica Mountains might be a possibility without the freeway and associated development as a barrier. *Sciurus niger* is common in the Simi Hills, particularly at the urban periphery (DSC, pers. obs.).

*Verdugo Mountains/San Rafael Hills*

Populations of *S. griseus* in both the Verdugo Mountains and the adjacent San Rafael Hills are isolated from the San Gabriel Mountains to the north by Interstate 210 and by dense residential development along Foothill Blvd. Despite searching promising areas such as La Tuna Canyon Rd., Crescenta Valley Park and the Whiting Woods neighborhood on the north slope of the Verdugos, and Descanso Gardens and Scholl Canyon in the San Rafaels, we could not locate any individuals during observational surveys in 2011–12. However, in approximately three months operating motion-activated cameras in 2012, we detected single individual *S. griseus* at two sites, one in Cedarbend Canyon and one near Whiting Woods, confirming that the species persists in the Verdugo Mountains. In the San Rafael Hills, Erkabaeva (2013) observed four *S. griseus* in a group on one occasion at Descanso Gardens in La Cañada, as well as several lone individuals here during 2012. Later, a motion-activated camera that had been placed at Descanso Gardens since 2012 recorded a single *S. griseus* in July 2014, indicating the persistence of at least a small population here. *Sciurus niger* is very common throughout both the Verdugo Mountains and San Rafael Hills, including within seemingly pristine habitat far from development (DSC, pers. obs.).

*Northeastern Los Angeles*

The hilly residential neighborhoods of Los Angeles just south of the San Rafael Hills (including Eagle Rock and Highland Park) appear to have also lost at least one lowland population of western gray squirrels. Several individuals were observed in planted pines in the upper portions of Forest Lawn Glendale on the Eagle Rock border in 1997 (DSC,

pers. obs.), but a check in 2011 (this study) revealed only *S. niger*. Lack of records from considerable time afield in this region and no sightings by naturalists based at the Debs Park Audubon Center in Highland Park (J. Chapman, pers. comm.) suggest that no current population of *S. griseus* persists in this area, which includes the lowermost portion of the Arroyo Seco.

#### *Pasadena/West San Gabriel Valley*

Small numbers of western gray squirrel occur at the northern/urbanized edge of Pasadena/Altadena (c. 457 m a.s.l.), where *S. niger* is now abundant. One was observed in 2011 (DSC) in the courtyard of an abandoned U.S. Forest Service facility adjacent to Hahamongna Watershed Park near the Pasadena/La Cañada border, and according to a local resident, up to four individuals, including a probable family group (in January 2011), have been recorded here in recent years (L. Paul, via email). *Sciurus griseus* is occasional in the more wooded residential neighborhoods along the northern tier of Altadena at the base of the mountains (e.g., near Eaton Canyon and Kinneloa Canyon), but has apparently abandoned locales slightly downslope in denser residential areas, including a former retirement facility (“The Scripps Home” at 2212 N. El Molino Ave.) that had its mature trees removed prior to a redevelopment effort in summer 2011 (an action which apparently drove out *S. griseus*, *vide* L. Paul). More significantly, a population of *S. griseus* that once occurred in remnant oak-walnut woodland amid residential estates along Mission Canyon at the border of San Marino and Pasadena (including Lacy Park) persisted to around 2012, with the last records (each of a single individual) being along Kewen Drive in San Marino on several dates in 2010 (J. Garrett, via email), and again in 2012 (M. Nakamura, CSULA web survey form). We also last received reports from the nearby Huntington Library around the same time (three separate sightings; T. Allison, CSULA web survey forms in 2008 and 2010; S. Claytor, photograph in 2008). We know of no remaining population of *S. griseus* here or along the lower Arroyo Seco south of Hahamongna/Devil’s Gate Dam.

#### *East San Gabriel Valley/San Jose Hills*

As in the west San Gabriel Valley, western gray squirrels occur widely in canyons and locally in residential areas in the foothills on the northern tier of the east San Gabriel Valley (e.g., above Monrovia, San Dimas and La Verne, >305 m a.s.l.). South of here, the low range of hills in the eastern San Gabriel Valley referred to as the San Jose Hills apparently serves as an ecological connection between the San Gabriel Mountains and the Puente-Chino Hills, which then connect to the much larger Santa Ana Mountains to the south (see Cooper 2000). Here, the species persists only at Bonelli Park (San Dimas) and in the “Industry Hills” near La Puente, and several extirpations have been very recent (e.g., observed by DSC at Walnut Creek Park in Covina in 2011 but not since; *vide* AEM).

#### *Puente-Chino Hills*

Western gray squirrels occurred in multiple canyons and open space areas from Diamond Bar and Rowland Heights west into Whittier and La Habra Heights, and south into Brea, and Chino Hills State Park during the late 1990s (DSC, unpubl. data). A population in Turnbull Canyon in the Whittier Hills (far western Puente Hills) was apparently extirpated in the late 1960s following a major fire that burned many mature oaks (J. Schmidt, in litt.), indicating that even by then some loss had occurred. By the late 2000s they had been extirpated west of Harbor Blvd., with replacement by *S. niger*,

including along Powder Canyon in Rowland Heights/La Habra Heights, where *S. griseus* was present in late 2005 (1, R. Erickson, unpubl. data) yet absent by 2007 (DSC, unpubl. data; *fide* L. Longacre). The latter location is particularly notable, as the canyon is directly contiguous to hundreds of acres of natural habitat, has been protected as part of the Puente Hills Landfill Conservation Authority, and has seen little if any land use change in the past 20 years. A devastating fire in 2008 that burned most of Chino Hills State Park resulted in the immediate loss of most western gray squirrel populations there, with only a very small number of individuals persisting in oak woodland in the remote center of the park, north of San Juan Hill (A. Ing, pers. comm.).

#### *Pomona Valley/Claremont*

While still present at Rancho Santa Ana Botanic Gardens and along the San Gabriel foothills through the northern portion of Claremont (e.g., San Dimas Canyon, Marshall Canyon, *fide* AEM), western gray squirrel has been recently extirpated from several areas, and replaced by *S. niger*, within the city of Claremont to the south, including the Claremont Colleges area (Guthrie 2009). There are apparently no historical or recent records of *S. griseus* from the eastern Pomona Valley nor along the lower Santa Ana River Valley upstream of Prado Dam.

#### *Redlands/Yucaipa (San Bernardino County)*

Western gray squirrels are widespread and conspicuous residents of the San Bernardino Mountains. However, lowland populations away from the lower foothills persist (as of 2014) at University of Redlands, Sylvan Park, Ford Park, and Prospect Park (Ortiz 2014). The species has also been reported in the “Sunset Hills” area of Redlands just south of Interstate 10 and in an apparently small area of Yucaipa (including Third St.), where they are found in mature pines in a residential area (CSULA web survey). These populations do not appear to be in contact with *S. niger* as of 2014, and are much higher in elevation than other lowland sites discussed. However, because they are persisting away from the main mountain ranges in what is still obviously lowland (non-montane or foothill) habitat, we have included them here.

#### *South Orange County*

In contrast to the report by Pequegnat (1951) that the western gray squirrel was not found in the Santa Ana Mountains, the species is present in several oak-filled canyons in the Santa Ana Mountains (e.g., Trabuco Canyon, CSULA web survey and J. Ortiz, via email; Modjeska Canyon/Tucker Wildlife Sanctuary, CSULA web survey; Whiting Ranch Wilderness Park, R. Hamilton, via email). Additional reports to the CSULA web survey locate western gray squirrels at the suburban-wildlands interface west of Lake Elsinore. Whether they are recent (post-1950s) arrivals to this range is not known.

Away from the Santa Ana Mountains, two small populations are known from Oak Canyon Nature Center in the Anaheim Hills, and along the “Santa Ana River canyon” where the Chino Hills meet the northern Santa Ana Mountains (AEM, unpubl. data; B. Leatherman, via email). We know of no records from the San Joaquin Hills, where *S. niger* has been present in residential areas since around 2010 (R. Erickson, via email). Like much of San Bernardino (and Riverside) County, *S. niger* has only recently (late 1990s) penetrated Orange Co., but it is now widespread and common into Irvine (D. Willick, via email).

### *Other Areas*

DSC (unpubl. data) observed a small number of what appeared to be western gray squirrels in pines at the golf course at the Palos Verdes Country Club near Malaga Cove on the Palos Verdes Peninsula in the 1990s; in this same area in roughly the same time period, a local naturalist observed what appeared to be a single individual in the same area (R. Melin, via email). A recent visit to this area (October 2014) revealed that it still supported a dense forest of coast live oak, toyon (*Heteromeles arbutifolia*), many mature planted conifers and eucalyptus, and a riparian strip running through the golf course (DSC, pers. obs.). And, whereas the eucalyptus plantation here has apparently been established for more than a century (Gales 1988), due to the extreme isolation of this area from any other known *S. griseus* populations, its coastal location, and the possibility that this population derived from deliberately introduced individuals (or pertains to the eastern gray squirrel, *Sciurus carolinensis*), we consider a Palos Verdes population to be “hypothetical” for now until more information is uncovered that would support its inclusion in the current range of the species.

### Discussion

Our investigation into the distribution of the western gray squirrel in the Los Angeles area elucidates its status as essentially a foothill species that is now rare and declining below around 457 m elevation, particularly in areas where it has come into contact with the eastern fox squirrel. Away from its main strongholds in the western Santa Monica Mountains, the San Gabriel Mountains, and the Santa Ana Mountains, small, isolated populations persist only in the Santa Susana Mountains, Griffith Park, the Verdugo Mountains and San Rafael Hills, the San Jose Hills, the Chino Hills, at Rancho Santa Ana Botanic Gardens in Claremont, and in Redlands/Yucaipa. Based on local naturalists’ observations, several lowland populations appear to have declined in the past five years, including that in Bonelli Park, the San Rafael Hills, Chino Hills State Park, and along the Santa Ana River Canyon near Yorba Linda. Invariably, extirpations have occurred concurrently with colonization by the ubiquitous *S. niger*.

It is probably unlikely that truly extirpated, isolated lowland populations in the area will re-develop on their own. Areas of recent extirpation (or near-extirpation, where *S. griseus* is no longer resident but may occur irregularly) are typically separated from the nearest presumed source population by more than a kilometer, and generally by dense residential or urban development. Multi-lane freeways now provide formidable barriers between these areas of extirpation and source populations of *S. griseus*. Remarkably, animals do persist in a handful of lowland areas with very limited habitat, including the Industry Hills in La Puente, which suggests that certain small, isolated subpopulations may act as “refugia”, perhaps from pathogens that periodically sweep through larger and more intact populations. Of course, these same refugia are vulnerable to their own extinction events, and so are almost certainly temporary.

Erkabaeva (2013) demonstrated that the length of projected coexistence of the two squirrel species in a given habitat fragment depends upon both the size of the habitat fragment and the structure of the habitat within the fragment, with length of coexistence associated with a higher diversity of food bearing tree species and coniferous trees. *Sciurus griseus* had a high probability of going extinct within a relatively short period of time (10 to 40 years) in small to medium-sized habitat fragments. The presence of the *S. niger* in the same habitat brought about extinction in a shorter period of time.

Competition with other squirrel species has been suggested as a potential cause of *S. griseus* decline (or a contributor to its current patchy distribution) in the region, but the mechanisms involved in this relationship need further study. Extirpation sites generally support very high densities of *S. niger*, yet this species simply occurs at higher densities in general. *Sciurus niger* is highly urban-adapted, and occurs at all the sites where *S. griseus* has vanished, and we have not confirmed a site where *S. griseus* has been extirpated and where *S. niger* is completely absent. Still, King (2004) found few interactions among *S. niger*, *S. griseus*, and even California ground squirrel (*Spermophilus beecheyi*) in her study area where all three occur in San Dimas, California (eastern Los Angeles Co.), and Ortiz (2014) also observed very few aggressive interactions between *S. niger* and *S. griseus* in her local study areas. Regardless of the mechanism, the loss of *S. griseus* in these areas – and region-wide – may be associated with a profound ecological change and degradation of seemingly healthy oak woodland and other habitat, particularly in wildland areas where replacement has occurred (e.g., the Puente-Chino Hills).

Larger wildland areas where *S. griseus* is persisting in the presence of *S. niger* are of particular interest because these appear to offer the basic habitat needs of both species, at least for some period of time, and possibly in different areas of the landscape. The discovery of nests of *S. griseus* well into protected open space such as in the rugged Cedarbend/Whiting Woods area of the Verdugo Mountains (DSC, unpubl. data) and at San Dimas Canyon Park (King 2004) suggests a pattern of edge-avoidance, possibly related to increased competition with the eastern fox squirrel at the urban edge. However, this pattern breaks down at sites like Fern Dell in Griffith Park, where *S. griseus* occurs a few feet from houses and dense urbanization (DSC, unpubl. data). Here, supplemental feeding or food provisioning may simply be “propping up” the population of *S. griseus* which has also been aided by the abundance of planted trees providing additional food sources (fruits and nuts). Although we have made a few direct incidental observations of supplemental feeding (e.g., unshelled peanuts dropped at Fern Dell in Griffith Park being carried off by *S. griseus*), it probably occurs widely. Other vegetative characteristics that allow *S. griseus* to persist here include some amount of closed-canopy woodland (or woodland-like groves of trees) with an open understory rich in non-woody debris and leaf litter; older, mast-producing trees for food; and at least a few very tall trees for nest placement (Linders and Stinson 2007), characteristics that still apply to many parks in the region.

More proximate factors in the decline of *S. griseus* relevant in our study area include death from injury and disease. Mortality from roadkill has been shown to be a major (if localized) factor in squirrel deaths in studies in Washington state (Linders and Stinson 2007), and *S. griseus* is frequently detected as roadkill in the Los Angeles area (pers. obs.). Many sites at the urban-wildland interface, including sites with documented *S. griseus* extirpations have roads along a canyon bottom, making squirrels that live in low densities and that forage on the ground particularly vulnerable. Other important causes of death and/or population decline include necrotic mange (found in many populations of *S. griseus* but oddly, apparently undocumented in the introduced *S. niger* in California, *per* King 2004); habitat quality decline from removal or disruption of the forest canopy due to development, tree-cutting, or fire; soil trampling and compaction (which reduces the biomass of fungi and perhaps other foods); and extreme natural events such as prolonged drought, which work synergistically to wipe out small populations. However, considering how modified the current habitat of many lowland *S. griseus* populations is (e.g., planted pines on golf courses), habitat transformation would seem to be a relatively minor threat.

Based on the continuing trend of extirpation in the region, we consider all existing lowland populations of *S. griseus* to be highly imperiled throughout the Los Angeles area. We estimate one of the largest intact populations within the urban core of the region, that at Griffith Park, at well under 50 individuals, and even here it is geographically limited within the park itself, with most of the population in two adjacent canyons (DSC, unpubl. data). Smaller, more isolated populations such as that at Rancho Santa Ana Botanic Gardens and at various patches in the San Jose Hills are now “landlocked” by freeways and urbanization and are probably much more imperiled; populations here and the Chino Hills are now surrounded and infiltrated by *S. niger* (*fide* A. Ing), and they may not be able to resist continued invasion by this species. In the case of Redlands/Yucaipa, it is likely only a matter of time before *S. niger* colonizes and saturates the residential areas and parks where *S. griseus* currently occurs alone.

Should re-introduction of *S. griseus* to lowland areas be attempted, we recommend this be limited to large, protected areas of natural habitat; however, reintroduction into areas where *S. niger* has already saturated the surrounding landscape and *S. griseus* has disappeared, such as at Franklin Canyon Park in Beverly Hills or along the lower Arroyo Seco in Pasadena, seems unlikely to succeed in the long term. Another possibility might be the modification of large closed landfills that have trees with a significant amount of closed canopy and that produce appropriate food items. We refer readers to Gatza (2011) for information on a Habitat Suitability Model that would support *S. griseus* while not being conducive to *S. niger*. Landfills within large urban areas often cover hundreds of hectares, and modification of portions of these landfills with corridors between suitable habitat fragments could provide new habitat for “lowland” western gray squirrels. We would not recommend introducing individuals from outside into areas of continued occurrence, such as Griffith Park, which would have the potential to introduce an unknown pathogen into vulnerable, isolated populations.

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## **A Young-of-the-Year Giant Sea Bass, *Stereolepis gigas* Buries Itself in Sandy Bottom: A Possible Predator Avoidance Mechanism**

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The adult giant sea bass, *Stereolepis gigas*, (GSB) is the largest teleost inhabiting California's shallow rocky reefs, attaining a length of about 2.3 m (7 ft) and a maximum weight of around 256 kg (563 lbs) (Baldwin and Keiser 2008). They range from Humboldt Bay, California to Oaxaca, Mexico, including the Gulf of California (Miller and Lea 1972). Adults consume a wide variety of prey and occupy rocky bottom habitat ranging from approximately 7–40 m (25–130 ft) of water (Miller and Lea 1972) and can forage over sandy bottom, away from rocky reefs (Baldwin and Keiser 2008). After their peak commercial catch in 1932, at just over 114,000 kg, the population quickly crashed and their numbers have remained depressed ever since; this has inhibited detailed research (Pondella and Allen 2008).

Young-of-the-year (YOY) GSB pass through various color phases and morphological changes during early development. These transitions help it to appear cryptic, while hiding to avoid predators during a vulnerable stage of life. When less than 2.5 cm (1 in), these YOY appear black with several small white spots around its face (Fig. 1). This black stage has very large black dorsal and pelvic fins, with transparent pectoral, anal, and caudal fins. The black juveniles morph through a “brown” stage, to a bright orange fish (Fig. 2). The black dorsal fin changes to orange, while the enormous pelvic fins remain black. Color expands outward to include half of the pectoral and anal fins, and the entire tail remains clear. The white spots remain from the earlier stages, and small black spots also appear (Pers. obs., and Benseman, unpublished data). These YOY appear to frequent open, sand and mud-bottomed habitat between 2–30 m (7–100 ft) for the first few months after settlement (Benseman, unpublished data).

During a focused SCUBA survey for YOY GSBs at Veteran's Park in Redondo Beach, Los Angeles County, California, Michael Couffer located a roughly 2.5 cm (1 in) long orange juvenile GSB in 5.5 m (16.5 ft) of water, floating upright in the bottom of a shallow sandy depression with its dorsal and pelvic fins closed. The bottom was clean sand without surface detritus. When approached, the GSB raised its dorsal and pelvic fins and left the depression, moving slowly within 30 cm of the bottom. The fish was photographed to record the sighting time in image metadata, and followed from about a meter away to acclimate the fish to human presence so that it could be photographed in profile.

After the fish had moved about 9 m, Mr. Couffer approached to half a meter to photograph its spot pattern. The fish startled, and darted toward the bottom at an angle. As the fish reached the bottom, it turned on its side at the last instant and buried into the soft sand by undulating its body like a flatfish. It pushed its head beneath the sand and undulated until the entire fish was buried in under three seconds. I took several photos of



Fig. 1. An 18mm YOY Giant Sea Bass from Newport Beach, California.



Fig. 2. A 75mm YOY Giant Sea Bass from Newport Beach, California.



Fig. 3. Scales on the side of the buried YOY Giant Sea Bass show through the sand to the left of the insert.

the exact spot where the GSB had disappeared (Fig. 3), and then put a 15 cm net over the spot, working the net's frame down into the sand. The fish remained buried. I dug my hand deep into the soft sand under the net, lifted a ball of sand containing the fish up into the net. As the sand fell away to the sides of the ball, the GSB burst out of the sand and up into the net. I measured the GSB at 32 mm, and released it. The GSB darted beneath me as I knelt on the sand. I pushed off the bottom, but the fish was gone.

The GSB's actions appeared to be a flight response, a possible last-ditch effort to evade predation in an area where there was no available cover for shelter. Unlike flatfish that may cover themselves with soft sediment to ambush their prey (Gibson and Robb 1991), or certain benthic gobiid fishes that have mutualistic relationships with shrimp that dig holes for shelters (Horinouchi 2008; Thacker et. al. 2011), this GSB was behaving as if actively trying to avoid detection by a "predator". Senioritas, *Oxyjulis californica*, are also known to bury themselves to avoid predators, but this occurs mostly at night, with the seniorita remaining buried for protection (Hobson, E.S. 1968), and not as an immediate escape response. This GSB predator evasion method should prove highly effective, as few predators could dig in the sand for the fish after burial. The bottom was so uniform that if the observer had looked away from the spot where the fish had buried, its location would have been lost (Fig. 3).

The open expanse of sand and mud bottoms that these small YOY GSB utilize make ideal nursery areas since there is an abundance of food, such as mysids and other arthropods (Dahl 1952), and relatively few inhabitants, including predators (McLachlan 1990). However, when a juvenile does encounter a predator, it must rely on its cryptic coloration and shape, and other types of active and passive predator avoidance strategies. The burying behavior observed may be a successful last-resort predator avoidance strategy for GSB, and is certainly the first one documented.

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## **Nelson's big horn sheep (*Ovis canadensis nelsoni*) trample Agassiz's desert tortoise (*Gopherus agassizii*) burrow at a California wind energy facility**

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Research on interactions between Agassiz's desert tortoises (*Gopherus agassizii*) and ungulates has focused exclusively on the effects of livestock grazing on tortoises and their habitat (Oldemeyer, 1994). For example, during a 1980 study in San Bernardino County, California, 164 desert tortoise burrows were assessed for vulnerability to trampling by domestic sheep (*Ovis aries*). Herds of grazing sheep damaged 10% and destroyed 4% of the burrows (Nicholson and Humphreys 1981). In addition, a juvenile desert tortoise was trapped and an adult male was blocked from entering a burrow due to trampling by domestic sheep. Another study found that domestic cattle (*Bos taurus*) trampled active desert tortoise burrows and vegetation surrounding burrows (Avery and Neibergs 1997). Trampling also has negative impacts on diversity of vegetation and intershrub soil crusts in the desert southwest (Webb and Stielstra 1979). Trampling of important food plants and overgrazing has the potential to create competition between desert tortoises and domestic livestock (Berry 1978; Coombs 1979; Webb and Stielstra 1979).

Native ungulates like Nelson's big horn sheep (*Ovis canadensis nelsoni*) co-occur with desert tortoises in portions of the desert southwest. Due to habitat and partial dietary overlap of various annual forbs and grasses at certain elevations (Ernst and Lovich 2009; Oehler et al. 2003), there is potential for contact between these species. Although there are data demonstrating damage and destruction of desert tortoise burrows caused by domestic ungulates (Nicholson and Humphreys 1981; Avery and Neibergs 1997), it is previously undocumented if native sheep like Nelson's big horn sheep are capable of similar impacts to tortoise burrows.

On 29 September 2013, we documented desert tortoise burrow collapse caused by Nelson's big horn sheep trampling at a wind energy facility in Riverside Co., California, USA (33°57'06"N, 116°40'02"W, WGS84). In the summer of 2013 (1 June 2013 to 14 November) 48 Reconyx and Wildlife motion sensor trail cameras were deployed at the entrances of desert tortoise burrows during an ongoing investigation of the effects of wind energy generation on behavior and activity of this species (Lovich et al. 2014). Cameras were mounted on 1.5 m foot tall steel stakes inserted into the ground approximately 1 m from desert tortoise burrow entrances that were known to be occupied or used recently. Cameras were activated by movement of wildlife via an infrared sensor,



Fig. 1. Active desert tortoise burrow collapse caused by Nelson's big horn sheep in a series of 4 motion sensor camera images.

and programmed to take 1–5 photographs at a trigger speed of 0.2 sec. Each month, an investigator checked each camera and downloaded photos onto a data storage device. Lastly, surface air temperatures were collected every 30 minutes from an onsite Remote Automated Weather Station (WWAC1; accessed via the MesoWest website (<http://mesowest.utah.edu/index.html>)).

Our motion sensor cameras recorded three Nelson's big horn sheep approach a north facing active desert tortoise burrow (previously occupied by an adult male desert tortoise on 13 June 2013) at 1140 h. From 1241 h to 1303 h, several Nelson's big horn sheep gathered below the entrance of the burrow, brushing loosened soil around the entrance of the burrow with their hooves, eventually causing the outer walls to collapse (Fig 1.). Three different Nelson's big horn sheep then proceeded to lie down and in the process compact the soil, rocks and sticks on top of the newly collapsed entrance from 1304 h to 1429 h.



Fig. 2. Nelson's big horn sheep at the entrance of desert tortoise burrow. Nelson's big horn sheep may have been eating tortoise feces or soil, or simply investigating the burrow.



Fig. 3. Domestic cattle walking past the entrance of a desert tortoise burrow.

Several Nelson's big horn sheep remained standing at the burrow from 1430 h to 1542 h. During these observations ambient air temperature ranged from 30.56 C to 32.50 C.

Over the course of the camera-trapping study, Nelson's big horn sheep were also recorded walking past or standing at the base of seven different desert tortoise burrows at various other locations throughout the study site. Photographs also revealed what appeared to be Nelson's big horn sheep grazing near the mouth of the burrow (Fig. 2). Since few plants grow in the mouth of active tortoise burrows, the sheep may have been eating soil or possibly the fresh feces of desert tortoises that are comprised mostly of partially digested grass and forbs. In addition to big horn sheep, domestic cattle were captured by motion sensor cameras walking past the base of four desert tortoise burrows (Fig. 3).

The images we recorded are the first documented evidence of Nelson's big horn sheep trampling a desert tortoise burrow and subsequently collapsing the outer walls of the burrow in the process. Nelson's big horn sheep employ various strategies of seeking shade and cooler soil for bedding (Cain et al. 2008), and it appears that north-facing slopes (location of collapsed tortoise-burrow) may provide such a site. Alternatively, previous studies of big horn sheep have documented extensive movement and occasionally large descents from mountain ranges to use mineral licks at lower elevations, as they provide sodium which is crucial to physiological functions (Bangs et al. 2005; Holl and Bleich 1987; Watts and Schemnitz 1985). Since most terrestrial plants have low concentrations of sodium (Weeks and Kirkpatrick 1976), Nelson's big horn sheep may be mining essential minerals brought to the surface by tortoises through excavation of their burrows (Ernst and Lovich 2009; Turner et al. 1984). One study demonstrating soil ingestion, or geophagy, by bighorn sheep (*Ovis canadensis*) in Alberta, Canada found their feces contained as much as 30% soil in some samples (Skipworth 1974). Ingestion of desert tortoise burrow soil may be important to Nelson's big horn sheep as it could be a source of certain minerals (Beyer et al. 1994). Lastly, we hypothesize that relatively high plant productivity at the site (Ennen et al. 2012b; Lovich et al. 2015) attracts ungulates (Oehler et al. 2003), both domestic and native to the study area. Moderate winter precipitation produces an abundance of annual food plants for both desert tortoises and big horn sheep at the study site.

Trampling and collapsing active desert tortoise burrows may entomb resident individuals (Loughran et al. 2011; Nichols and Humphries 1981), although in the majority of observed burrow collapses at the site, tortoises were able to excavate

themselves (Loughran et al. 2011). In light of our observation, trampling may have greater impacts to slope dwelling rather than valley dwelling desert tortoises. Furthermore, female desert tortoises nest at the entrance and within burrows (Agha et al. 2013; Ennen et al. 2012a); consequently, trampling may negatively impact tortoise egg clutches or entomb emerging neonates (Berry 1978). Entombment of desert tortoises within burrows can cause physiological stress to the animal (Loughran et al. 2011), thereby leading to potential mortality (Lovich et al. 2011). We are unaware of any cases where bighorn sheep behavior resulted in mortality of desert tortoises and suspect that such interactions between the species are rare in comparison to interactions involving domestic ungulates.

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